

Open University Graduates

FROM all appearances the Open University seems to have weathered the storms of the first three years of its existence and the awarding of the university's first degrees recently set the seal on the formative years. But in spite of this first outward sign of success the critics of the university have not been silent and they have taken this opportunity to analyse the development of the university since the concept was first mooted and, not surprisingly, have come up with the conclusion that the way the university now operates does not completely conform with the much publicized principle enunciated in the mid-1960s that the Open University would educate the masses through the medium of radio and television.

It is not clear whether the label "a glorified correspondence college" which has been attached to the university recently, was issued in sorrow or in glee but it is misleading to think that the Open University can be compared with courses run by local education authorities. It must be admitted, however, that the greater part of the instruction of the university is provided in the form of course units which must be read weekly but these are supplemented and complemented by tutorial and counselling evenings as well as by radio and television programmes which taken together with the annual residential summer school build up into a unified structure which makes the Open University teaching system unique. With hind sight it seems inevitable that the university has had to rely on correspondence courses to provide the bulk of the instruction but the high quality of these courses has, in no small part, helped to put the emphasis where it lies today. But with this record of success behind it, the university, now considering its plan for the next three years, must decide whether the overall balance of teaching by correspondence, instruction through tutorials and enlightenment through the media is the most efficient and proper way to educate its students.

But what will the future bring? The emphasis so far has been very much on the undergraduate courses but some post experience courses have made their appearances in the last year. Will the university make an effort to remain primarily a vehicle for providing a second chance for students who for one reason or another did not progress to university (or some other degree granting institution) at the age of eighteen or will it concentrate more and more on providing refresher courses and other courses for already qualified people?

It is, of course, possible that in the not too distant future the university will find itself being forced to change its courses simply because of the number of applications it receives for particular types of study but should it in such circumstances bow to these pressures or should it make all efforts to go out and search for the type of students it wants? One of the admitted deficiencies of the present student body is that it contains too few students who are employed in jobs which can be classed as manual, or to put it bluntly the university is not succeeding as well as it should in attracting students

from the working classes. Certainly 4% of the present students are working class, a figure which compares most favourably with the number of children of working class parents studying at conventional universities in Britain. But if the university is to make the mark it should on British society then the difficult task of increasing this percentage must be attacked vigorously. This is not easy, for the motivation for non-manual workers to obtain degrees is higher than for manual workers.

In the short term, however, the university should be thinking of how best to cater for the needs of its present student body. The great majority of these who started with no formal qualifications are completing one course a year which means that they are now after three years half way towards their coveted degrees. Many of these students who are sacrificing a great deal of their family lives would welcome the opportunity to accelerate their progress. Would it not be possible to arrange a system by means of which students after they had demonstrated their ability by successfully studying for three years could then spend the best part of a year at a more conventional university obtaining the remaining necessary credits towards their degrees? Whichever way the Open University turns in future it must make closer ties with conventional universities. The drive and initiative which got the university off the ground is still present but it now needs to be channelled towards different goals.

100 Years Ago



AFTER the alarming rumours that have recently found their way into the newspapers, it is a great relief to receive what appears to be really authentic news of the safety of Sir Samuel and Lady Baker. It appears, from the message received by the *Daily Telegraph*, that they arrived at Kiartoim on the 29th of June. It is stated that the party had been as far south as a place called Mosindi, near the chief village of Kamrasi, the King of Unyoro, which would be in about 1°45' N. lat., and about 80 miles to the east of the shores of the Albert Nyanza. Here Sir Samuel is said to have been attacked by a chief named Kabriki, and, on his retreat, by a party of slave hunters. He seems to have established another Egyptian station at a place called Fatiko, somewhere to the south of Gondokoro. The story about the Albert Nyanza and Lake Tanganyika being one, which forms part of the news published by the *Daily Telegraph*, is certainly very startling news, and must at present be received with great caution, though the *Telegraph* correspondent declares he received it direct from the lips of the Emancipator of Central Africa himself.

From *Nature*, 8, 209, July 10, 1873.



OLD WORLD

Disarray at the International Whaling Commission

CALL my bluff was the name of the game at last week's meeting of the International Whaling Commission. But it may be ninety days before the results are known.

By the end of the somewhat fraught meeting, the proposal for a ten year moratorium had gone by the board, the plans for a permanent secretariat had been shelved, and attempts to get the non-IWC whaling nations to join had received no reply. The commission had passed its toughest resolution ever, cutting the fin whale quota in the Antarctic by 500 to 1,450 for next year, and providing for a complete moratorium on fin whaling in three years time, but the USSR and Japan—the chief whaling nations—had voted against it and are likely to object to the resolution. Under the commission's rules they have ninety days to lodge an objection. If they do so, they are not bound by the commission's decision, although they can still remain members of the IWC.

It was a tough week. The scientific committee, which met the week before the IWC convened, had a hard time finding agreement on its recommendations, and when the plenary session started it contained some of the most combative arguing the commission has seen for a long time. The United States did its best to discredit the work of the scientists and push for a complete moratorium on a safety first basis, finally forcing Japan and the USSR into a corner over the fin whale.

Dr Robert White, Director of the United States National Oceanic and Atmospheric Administration and head of the United States delegation, said in his opening address that "science can

only provide facts and illuminate the probable consequences of various courses of action. Unfortunately, in the case of whale stocks the scientific 'facts' are questionable at best. The data on which the catch quotas of various species of whales are set have a large and unknown measure of uncertainty, and our scientific understanding of the population dynamics and ecology of whales is woefully weak . . . how many realize that the yield calculations are based on population models which in turn are based on uncertain assumptions—but on very little real data?"

The proposal for a complete moratorium went down eight votes to five (the Soviet Union, Japan, Norway, South Africa and Iceland against, Denmark abstaining), and quotas for the remaining stocks were set, although the meeting witnessed the first open split between the USSR and Japan who between them account for eighty per cent of the whales taken. Japan wanted a catch quota of 12,000 set for minke whales in the Antarctic, but was outvoted 13 to 1 in favour of a 5,000 catch. Quotas for other areas and stocks were either reduced or stayed the same where the scientists held that stocks were plentiful, but the real crunch came over the fin whale.

The fin is the largest of the baleen whales still hunted, but its Antarctic population is only one third of the size needed to obtain a maximum sustainable yield. Proposals that this year's catch should be reduced to 1,450 met with approval from the Soviet Union which also agreed to phase fin whaling out over the next three years. But Japan, while agreeing to this year's

quota, would not agree to the eventual moratorium. The United States insisted on putting the motion to the vote with both clauses in one motion. It was passed seven to two with five abstentions, with, not surprisingly, Japan voting against, supported by the Soviet Union.

During the course of debate both countries threatened to leave the commission. The United States effectively called that bluff, but it is more than likely that the two countries will object to the resolution, which will mean that there is no fin whale quota set for the Antarctic this year.

Mr Inge Rindall, chairman of the commission, said last week that the opponents of whaling "have perhaps been a little too enthusiastic, especially as what we have gained is so small". If the Antarctic fin whale was left unhunted, Mr Rindall said, it is estimated that it would take 27 years for it to grow to its maximum sustainable yield; if 1,450 whales were taken each year it would take 35 years to reach that level. "Pressure groups" Mr Rindall said, "have had a most helpful effect on the work of the commission and have led to the reduction in quotas, but I am afraid that by pushing this affair too far it has had a counterproductive effect".

The United States, however, sees matters differently. Dr Lee Talbot, a senior adviser to the Council on Environmental Quality said last week that the resolution is "a most significant test of whether the commission exists to serve the industry or to serve the world through the proper management of the world's whale resources: and we have seen a dramatic development of support on this issue culminating in a vote in which the only opposition was from the Soviet Union and Japan".

In fact the situation may not be too black. The two nations are likely to object, but it is more than probable that both will remain within the 1,450 quota that they were willing to agree before the moratorium pressure was applied. World public opinion is not an entirely negligible force.

The resolution has simply demonstrated that the commission is as toothless as it has always been known to be. Largely in pique at being put on the spot Japan has refused to put up money for a permanent secretariat for the commission, although the matter will come up for discussion again next year, and the USSR has suggested that the International Observer Scheme,

Table 1 Whale Quotas for 1973–74

Area	Species	Quota	1972 Catch *	1972 Quota
Antarctic	Fin	1,450	1,761	1,950
	Sei	4,500	3,859	5,000
	Minkie	5,000	5,745	5,000
North Pacific	Fin	550	758	650
	Sei	3,000	2,327	3,000
	Sperm (male)	6,000		6,000
	(female)	4,000	6,32 3‡	4,000
Southern Hemisphere †	Sperm (male)	8,000		8,000
	(female)	5,000	6,492 ‡	5,000
Total		37,500	33,781 §	

* Source: Bureau of International Whaling Statistics, Norway.

† Southern Hemisphere further subdivided into three areas: 60° W–70° E (1,900 males, 1,800 females), 70° E–170° W (2,900, 2,100), 170° W–60° W (3,200, 1,100).

‡ Catch figure includes both sexes.

§ All large whales caught in all areas by all countries (including non-IWC members). Species only protected in areas specified above. For example fin whales are not protected in North Atlantic where 598 were taken last year. 1971 catch approximately 41,000 *in toto*.

implemented last year after 14 years of negotiation will not be put into effect again this year.

All the commission has done is demonstrate that it is easy for those nations that no longer whale to wear their consciences on their sleeves. It is harder for them to persuade the nations who see whaling as economically attractive to feel the same way.

SPACE

Snags with UK-6

THE Science Research Council has agreed plans for a UK-6 scientific satellite and approval of the scheme now rests with the Department of Education and Science.

If approved, the satellite will carry three experiments, one from the University of Bristol to study heavy cosmic rays, one from the University of Leicester to investigate time variations in X-ray sources following up the work of OAO, and a joint experiment from the University of Birmingham and Mullard Space Science Laboratory to examine soft X-ray sources with the help of collecting mirrors (see *Nature*, **241**, 403; 1973).

Approval for the satellite has been somewhat delayed by procedural problems in the Ministry of Defence Procurement Executive which manages the contracts for the SRC. The satellite is intended to be relatively cheap—total cost is put at about £3.9 million including the launch—and full advantage will be taken of experience gained from work on UK-4 and X-3. As a result, tendering for the contract was originally going to be on a single tender basis, but the Procurement Executive decided instead to have a competitive tender between the British Aircraft Corporation and Marconi Space and Defence Systems. Unfortunately one of the companies was unwilling to bid on a competitive basis and a certain amount of argument ensued. Tenders are expected, however, about September, by which time the DES should have made its decision.

SELECT COMMITTEE

Commission Won't Talk

THE Select Committee on Science and Technology has had to postpone next week's trip to Brussels to take evidence from the European Commission.

Mr Airey Neave, chairman of the select committee, said this week that the European commissioners have declared themselves unwilling to give formal evidence to the committees of national Parliaments. At present they maintain that they are only answerable to the European Parliament by way of the Council of Ministers.

Mr Neave said that it is likely that

other select committee chairmen will join him in demanding that the commissioners be liable to give evidence to national Parliaments on matters that affect national policies—for example, in the science and technology committee's case, on computers.

The problem is partly that the European Parliament has no machinery for taking formal evidence in the way of the House of Commons committees. Certain other national Parliaments have similar arrangements—the Bonn assembly for example—but it is not known if they have taken formal evidence in the way that British MPs are anxious to do.

The matter is likely to be taken up with British ministers, and may have to go to the European Council of Ministers for a decision.

The commission has, however, extended an olive branch in the shape of an invitation to visit Brussels informally while the matter is being sorted out. It appears that the commission is willing to talk to the committee off the record, but Mr Neave said this week that his

committee has yet to decide if it is prepared to go on that basis. The Overseas Aid select committee, however, has already accepted an invitation to make an informal "educational" visit.

Awkward though the matter is for the Science and Technology Select Committee, the attitude of the commissioners hits other select committees even harder. The Select Committee on European Secondary Legislation for example, is going to find it very difficult to do anything at all if the European Commissioners will not talk to it.

As a result it is possible that the chairmen of all the House of Commons Select Committees will act together in an attempt to resolve the problem.

If the visit had gone ahead as planned, the select committee would have taken evidence on computers, energy policy, nuclear power and research and development policy in the course of a two day visit to Brussels.

Some announcement on the committee's request to Mr James Prior that it be allowed to appoint a full-time science adviser is expected shortly.

Short Notes

Research and Development

MR JOHN DAVIES, Chancellor of the Duchy of Lancaster, is to be the minister responsible for co-ordination of research and development policy.

Previously Lord Jellicoe as Lord Privy Seal had held the responsibility, but with his resignation the situation has been reviewed and Mr Davies, rather than Lord Windlesham, the new Lord Privy Seal, is to have the responsibility. The reasoning behind Mr Heath's decision is that Mr Davies has responsibility for European affairs, and much research is now undertaken on a European basis.

Swine disease controls

MINISTERIAL orders to further control the operation of swill plants should be introduced by the end of August (see *Nature*, **242**, 4; 1973). The recurrence of swine vesicular disease on May 31, after a lull of six weeks from April 13, has been followed by eight more cases of the disease, five of them since June 28. Only one of the cases in the secondary outbreak occurred on a swill fed farm, and although links have been traced between several of the other outbreaks, ministry officials are not yet sure why the disease emerged again. But close control of new outbreaks, the new controls on swill plants, and a warm summer which will limit the activity of the virus should ensure that the disease will be fully controlled by the winter. Since December 11, 1972, when the disease was first confirmed, 50,000 pigs have been slaughtered in over 90 outbreaks.

Cannabis and Research

THE legalization of the smoking of cannabis for research purposes has hardly caused a stir in Britain. The new regulation follows in the wake of similar legislation in the United States that legalized the use of cannabis for research purposes in October 1970, but the British action is as much a tightening up of the law as a relaxation. Previously cannabis could be given by mouth by any qualified doctor although under no circumstances could it be smoked. Now a licence is required before it is used in any way for research purposes. Nobody has any immediate plans to take advantage of the new regulations. Until the quantities of cannabis in the blood can be measured, there is relatively little point in experimenting with human patients.

Nuclear Power Company formed

THE National Nuclear Corporation is the name of Britain's new nuclear power company. The corporation is to replace the two existing consortia as the contractor for building Britain's nuclear power stations. GEC is the chief shareholder with a 50 per cent holding, with the United Kingdom Atomic Energy Authority holding 15 per cent of the shares on behalf of the government. The remaining 35 per cent will be held by industry.

The corporation is to have an operating company, called the Nuclear Power Company, which will also be chaired by Lord Aldington. Directors for the two companies will be appointed shortly.

NEW WORLD

President Nixon takes a Firmer Stand on Energy

by our Washington Correspondent

JUST two months ago, President Nixon informed Congress that the United States was facing an "energy challenge" which required little more to set right than some tinkering with import and price controls. But by last week, his assessment of the situation had changed. He told Congress that the energy shortage is now "one of the most critical problems on America's agenda", and he put forward a set of much more radical proposals for dealing with it.

Among the new measures is \$100 million in new money for energy research with the promise of much more to come, a proposal to establish a new energy research and development agency modelled around the laboratories of the Atomic Energy Commission, a conservation drive aimed at reducing consumption of energy in the United States by 5% over the coming year, and the appointment of Governor John A. Love of Colorado to the directorship of a new energy policy office in the White House. The new proposals have met with considerably more enthusiasm than the previous set, but they still leave many questions unanswered.

The proposal which has met with almost unanimous approval is the move to increase funding for energy research and development. President Nixon's intention is to add \$100 million to his original proposal for \$772 million to be spent in the 1973-4 financial year on research into new sources of energy. But he also made clear that it will be just the first instalment of a programme which will soak up \$10,000 million over five years, beginning in the 1974-5 financial year. At least half of the \$100 million is earmarked for coal research, while the rest will be split between advanced energy conversion systems, environmental control, geothermal energy, conservation and gas cooled nuclear reactors.

The list of projects will at least please those who have been arguing that the

government's energy policy is at fault for putting too much money into the fast breeder reactor at the expense of other projects. And it may also help to erode Congressional support for a bill sponsored in the Senate by Senator Henry M. Jackson, which calls for expenditure on energy research and development of \$2,000 million a year over the next ten years (see *Nature*, **242**, 224; 1973). But the message is vague about where the money will come from since President Nixon says only that it must be found within the overall budget ceiling, presumably by taking it away from a less deserving cause.

The plans for splitting up the Atomic Energy Commission (AEC) to create the new Energy Research and Development Administration, and for establishing a new cabinet-level Department of Energy and Natural Resources, have also provoked little opposition so far, although they have prompted many questions. As described in the message, the Department of Energy and Natural Resources will take over all the functions of the Department of Interior except for those concerned with energy research and development, and the natural resource functions of other departments including the National Oceanic and Atmospheric Administration, lock, stock and barrel.

As for energy research and development, the idea is to split off the regulatory and licensing functions of the AEC and to use the remainder—chiefly the national laboratories—as the nucleus of an Energy Research and Development Administration (ERDA), which would also take in the energy research and development programmes of the Department of Interior. "The scientific and technological resources of the AEC should provide a solid foundation for building a well-conceived and well-executed effort", President Nixon said.

But it seems that ERDA will have a few strangers in the pack, for it will also contain the weapons and physical research programmes of the AEC. There has recently been talk of turning nuclear weapons production over to the Department of Defense, but that would require an amendment to the Atomic Energy Act which would negate the original intention to keep nuclear weapons firmly under civilian control. Such a move is, however, still on the cards.

What are the chances that the plan will be approved by Congress? First, it has some points which favour its passage

through the Congressional mill, including the support of Congressman Chet Hollifield of California, Chairman of the House Government Operations Committee to which it has been referred. But it also has some points against, including considerable confusion about whether or not it requires an amendment to the Atomic Energy Act and hence the approval of the powerful Joint Committee on Atomic Energy. Whatever happens, it is not clear whether the Joint Committee will continue to have oversight over ERDA and the present licensing and regulatory functions of the AEC (which would remain under the control of five commissioners). Also in the air is the question of who will take over the directorship of ERDA if it is given the green light from Congress, but there is little question that the present chairman of the AEC, Dr Dixie Lee Ray, is the strongest contestant at present on the books.

Congressional sources predicted this week that there is little chance that the reorganization plan will be approved by Congress this year. The House Government Operations Committee is, however, planning to hold preliminary hearings on July 24-26.

As for energy conservation, President Nixon has set targets for reduction of energy consumption throughout the United States of 5 per cent over the coming year, and a separate target of 7 per cent for the federal government. But he has provided no stick, such as gasoline taxes or penalties for large automobiles, to enforce the reduction. As for the federal government, the chief energy consumer is the Department of Defense, which will in any case cut down its fuel consumption when it is forced to stop bombing Cambodia. Federal departments have until July 31 to report on the specific steps they will take to meet the target. But even without any measures to force reduction of private consumption of energy, President Nixon's forceful exhortations to curb demand represent a considerable improvement over the mild statements that characterized his previous message.

Finally, Nixon's choice of Governor Love to take charge of the White House Energy Policy Office is also likely to placate his critics. A liberal Republican who has established a reputation for independence, Love has been given broad powers for formulating and co-ordinating energy policies. His appointment does not require confirmation by Congress.

Nature Subscriptions

THE *Nature* subscription list has been computerized. Each address label now carries a reference consisting of three letters and four numbers. Any queries relating to subscriptions should in future quote this reference.

HIGHER EDUCATION

Carnegie Reappraisal

by our Washington Correspondent

THE Carnegie Commission on Higher Education has come up with the eminently sensible suggestion that federal funds for science should be provided more steadily than they have been in the past, and that research funds for the social sciences, humanities and arts should be substantially increased. Unfortunately, however, the report fails to provide much justification for those suggestions, putting them forward as an article of faith. And that is unlikely to cut much ice with the keepers of the Federal government's purse strings.

The recommendations are contained in the latest of the commission's analyses of US higher education, a look at the purposes and performance of American colleges and universities. (*The Purposes and the Performance of Higher Education in the United States*, McGraw-Hill Book Company, \$2.45.) The chief thesis of the report is that higher education in the United States is going through a period of reexamination almost as intense as that of a century ago, when the universities first started expanding and began to include research and service to society as part of their functions. The commission believes that the performance of the universities in some areas, for example, in advancing human capability in

society at large, has been superior, whereas they have failed in other areas, for example, in enlarging educational justice for the postsecondary age group as a whole.

The value of university research "while clearly substantial, is impossible to calculate with any precision", the report states. The commission does, however, quote one study which comes up with the unusual measure that advances of knowledge have, during the past few years, contributed almost a quarter of the growth of total national income and more than one third of the increase of national income per person employed. "Such contributions require an adequate and steady supply of research funds, particularly from the federal government", the commission states, and considers "quite short-sighted" the recent reductions in funds available for basic research in the universities.

The commission clearly comes down on the side of those who argue that research funds should be distributed on the basis of productivity and proven excellence, rather than to meet arbitrary geographical criteria, for it recommends that "funds for basic research should be concentrated on highly productive centers and individuals". It also suggests, without any argument to justify the suggestion, that federal research funds spent in the universities should climb back to the level of about 0.3 per cent of gross national product, the only stated rationale being that such a level prevailed in 1967-68, the year of peak funding for science and technology.

Such recommendations are unlikely to evoke much dissent, at least in the universities, but the commission treads on more controversial grounds in its analysis of the place of secret research on campus. It flatly states that "all secret research should be eliminated from all campuses as a matter of national policy, except under quite unusual circumstances". The rationale behind that suggestion is that secret research is at odds with the inherent nature of academic life, "for secrecy is abhorrent to the search for truth when results must be open to analysis and comment to test whether they be truth or not". Aside from that suggestion, however, the commission misses a good chance to shed some philosophical light on the subject of secret research, and in particular it does not provide any discussion of where secret research should be carried out.

It can, however, be argued that the report is an overall look at the purposes and performance of higher education, and that it is not designed to provide a blueprint for university development. That being the case, the final chapter, discussing possible future trends in

higher education, should be the most interesting. In short, the commission argues that the universities are subject to two opposite forces. One, largely internal to higher education, is the pressure to return to what the commission calls an "emphasis on values", and "an adventure in trying to shape society—not be shaped by it". The other is the move towards closer ties to society, with public authorities increasing their ties to higher education through research funding, student support and so on.

It is no surprise to find that the report suggests that the latter force will prove to be the stronger. Nevertheless, the commission sees no "special problems for the continuation of 'pure scholarship' provided academic freedom is protected against internal and external attacks upon it, and a reasonable supply of resources is available".

Training Grants Saved

BIOMEDICAL scientists have won at least a partial victory in their long and vocal fight to keep alive the training grants and fellowship programmes of the National Institutes of Health. Dr Charles C. Edwards, the Federal Government's top health official, told Senator Edward M. Kennedy's Health Subcommittee last week that the Administration is reconsidering its plans to phase out the programmes. A decision on how much money will be available for them in the 1974 fiscal year (which began on July 1) is expected soon.

The Administration's proposal to phase out the training and fellowship programmes was announced in January, when the budget was unveiled, the chief argument used for ending the programmes was that since funding levels for biomedical research have levelled off, there is no longer any need to train large numbers of young medical scientists. The laws of supply and demand should be sufficient to ensure that the numbers of new scientists are matched to the numbers of jobs available.

But those arguments have been bitterly attacked by groups such as the National Cancer Advisory Board, the Association of American Medical Colleges, the Federation of American Scientists and many individuals. The day before Edwards announced the reprieve, for example, a group of scientists took the opportunity during hearings on medical ethics held by Kennedy's subcommittee to argue for the programmes to be reinstated because of the danger of losing good scientists.

NSF takes the Reins

A SMALL science and technology policy office has been established in the National Science Foundation to provide support for Dr Guyford-Stever in his new role as science adviser. Dr Russell Drew, a former member of the staff of the Office of Science and Technology who is now head of the Office of Naval Research Branch office in London, has been appointed head of the office. He will be joined by at least four of his former OST colleagues, Daniel De Simone, Edward J. Burger jun., F. Gilman Blake, and Hylan B. Lyon jun. The functions of the OST were formally transferred to Dr Stever at the end of June.

The final size and budget of the Science and Technology Policy Office have not yet been decided (the NSF budget for 1974-5 contained no money or staff positions specifically for the new office), but Dr Stever is due to testify before the House Committee on Science and Astronautics on July 17, so presumably the details will be worked out by then.

NEWS AND VIEWS

Very Long Baseline Interferometry

HIGH angular resolution observations have made an important contribution to radio astronomy in recent years. Specialized techniques have been developed to combat the relatively poor inherent resolution of the single radio telescope, and the two-element interferometer introduced into optical astronomy by Michelson in 1920 has been taken over into radio astronomy with such success that the resolution is higher at radio than at optical wavelengths in spite of the difference of a factor $\sim 10^5$ in wavelength. Even Hanbury Brown and Twiss's improvement on Michelson's angular resolution to 0.0005 arc s has been superseded by radio developments.

These very long baseline (VLB) radio interferometers which now span the continents and the oceans of the Earth present novel technical problems. Whereas the earliest short baseline interferometers measured coherence between the signals received in the telescopes using cables and radio links, the VLB approach has found a solution to the coherence problem by using two entirely independent receivers. The method involves the construction of receivers with very accurate frequency control; this is now possible with atomic frequency sources such as the hydrogen maser or rubidium standards. These provide the necessary phase stability for measuring coherence and at the same time they act as high precision clocks (accurate to better than a microsecond) which give the required synchronization of the tape recordings of the astronomical signals measured at each end of the baseline.

VLB interferometers have been operated between large telescopes in Australia, Canada, Sweden, Britain, the United States, and the Soviet Union. The longest baseline used so far was between the 22-m radio telescope of the Crimean Astrophysical Observatory at Simeiz, USSR, and the 37-m radio telescope of the Haystack Observatory at Westford, Massachusetts, a distance of 7,350 km (*Soviet Astr.*, **16**, 379; 1972). At the operating wave-

length of 1.35 cm the resolution was 0.0002 arc s with a baseline of 550×10^6 wavelengths.

On page 18 of this issue of *Nature*, Legg *et al.* report VLB observations between the 45-m radio telescope of the Algonquin Radio Observatory, Ontario, Canada, and the 25-m radio telescope at Chilbolton, England, operated by the Radio and Space Research Station. At the observing wavelength of 3 cm the 5,265 km baseline corresponds to 187×10^6 wavelengths. Legg *et al.* report observations of 3C84, the radio source associated with the 13.3 mag galaxy NGC1275; it is a Seyfert galaxy with an eruptive nucleus. At radio wavelengths this galaxy has three components, one 4 arc min in diameter comparable with the optical object, one ~ 10 arc s diameter and the one studied here which is found to be contained within 0.01 arc s. Detailed analysis of their observations suggests that this nuclear component may consist of four separate sources each about 0.001 arc s in diameter spread 0.006 arc s along a north-south line. Similar compact components are found in many extragalactic sources, including both radio galaxies and quasars. Kellermann and Pauliny-Toth (*Astrophys. J. Lett.*, **155**, L71; 1969) state that more than half of all sources observed at centimetre wavelengths are opaque radio sources with positive spectral indices. Such sources are, like the nuclear component of 3C84, extremely compact objects with high brightness temperatures in the range 10^{11} to 10^{12} K.

The interesting new result found by Legg *et al.* is that the 3-cm brightness temperature of the 3C84 components is 3×10^{11} K. This is close to the upper limit of brightness temperature allowed (10^{12} K) in a radio source; above this temperature inverse Compton scattering causes catastrophic energy loss. The magnetic fields in these components of 3C84 are estimated to be 10^{-2} to 10^{-1} gauss, among the highest values found in a synchrotron radio source.

R. D. D.

T-Cell Function and Experimental Arthritis

THE experimental models of arthritis which at present are used to elucidate the pathogenetic mechanisms of rheumatoid arthritis are those which set up opportunities for immune reactions and their sequelae to occur in the joints of prepared animals. In the case of adjuvant arthritis—inducible in rats by remote injection of water-in-oil emulsion incorporating killed mycobacteria—it has been, at least tentatively, suggested that the latter or their fragments constitute the antigen which, conveyed by histiocytes to the synovial membrane, there interacts with circulating specifically-sensitized T lymphocytes to produce the inflammatory changes of the arthritis.

The experimental disease is certainly transferable to untreated syngeneic recipient rats by thoracic duct cells from donors which have been relatively recently sensitized, and it is also reported that administration of anti-rat lymphocyte serum during immunization prevents the arthritis (Currey and Ziff, *Lancet*, ii, 889; 1966), so that cell-mediated immunity seems to be important in

its production especially as, in contrast to rheumatoid arthritis, local immunoglobulin synthesis by the inflamed synovium is not a feature (Cook and Jasin, *Arth. Rheum.*, **15**, 327; 1972). Nevertheless, depletion of T cells produced by neonatal thymectomy does not confer protection against arthritis developing on subsequent standard challenge.

Lennon and Byrd (see page 38 of this issue of *Nature*) have now adduced new evidence that although neonatal thymectomy on the one hand protects rats receiving basic myelin protein in an immunization schedule otherwise appropriate to induce auto-immune encephalomyelitis against developing the latter, on the other it induces an undue proneness to what looks like adjuvant arthritis, with histologically typical mononuclear cell synovial infiltrates. What may be relevant here, however, as Lennon and Byrd point out, is the fact that the worst arthritis was seen in the rats with the lowest pre-immunization blood lymphocyte counts and the least

antibody production after immunization with basic myelin protein. It looks as if, whatever the role of cell-mediated immunity in adjuvant arthritis, T-cell depletion by thymectomy enhances the likelihood of arthritis on this immunizing schedule through an effect on humoral responses. Incorporation of another antigen blocks the effectiveness of mycobacteria emulsions in inducing adjuvant arthritis, so Lennon and Byrd's explanation of their chance finding is that when T-cell numbers or function are depleted, the resulting impairment of capacity to form antibody to thymus-dependent antigens favours continuing antigen excess over antibody with consequent formation of soluble circulating immune complexes capable of mediating tissue damage.

There are several gaps in the argument, but it is at least possible to test the entailed hypothesis that the presence of complement-binding immune complex deposits in rheumatoid synovial membrane and in rheumatoid synovial fluid cells should correlate significantly with indices of diminished T-cell function. E. J. H.

ENZYMES

Open and Shut Case

from our Molecular Biology Correspondent

VOLUMES could by now be written about the conformation of NADH, both free and on proteins. Broadly speaking there are two possible states—open, with the two rings splayed out, and shut, with the rings folded on each other. Now two states are one too many, and much of the heavy artillery available to physically minded biochemists has consequently been brought to bear on the modest endeavour to establish which state prevails. Well before high-resolution nuclear magnetic resonance had begun to make its impact on biochemical problems, it was largely agreed that free NADH in solution must be in the closed configuration. This in any case best conforms with the tenets of nucleotide chemistry, which lay it down that nucleotides are adhesive molecules with a strong tendency to stack on each other (as in helical polynucleotides).

This conclusion was further strengthened by the observations of ring-current shifts, arising from the perturbation by the π system of an aromatic ring of the signals from protons lying close to it. This far then there was total accord. As to the state of the NADH (or NAD⁺) in its cofactor role on a dehydrogenase, there are spectroscopic argu-

ments to favour an open configuration, but the NMR results of Sarma and Kaplan, based on what looked again like ring-current shifts, persuaded them that the opposite was the case, and that on lactate dehydrogenase at least, the NADH was in the closed configuration. At this point, however, and not for the first time, the crystallographer irrupted tactlessly onto the scene with a 5 Å structure from Rossmann and his group of dogfish muscle lactate dehydrogenase, which showed all too plainly the cofactor in the open form.

Rossmann and his colleagues have now refined their structure further, and at a resolution of 2.8 Å they are able to make more detailed inferences about the state of the cofactor, and of its analogues, on the enzyme. At the same time Lee, Eichner and Kaplan (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1593; 1973) have had a new look at the NMR of NADH and NAD⁺ in the presence of a number of dehydrogenases, and have brought their interpretation into line with crystallographic fact. Lactate dehydrogenase from chicken and lobster, and the structurally related malate

dehydrogenase, all induced similar shifts and broadening of the lines in the NADH spectrum. Very small downfield shifts are observed in the adenine ring protons. In yeast alcohol dehydrogenase, by contrast, these signals move upfield. The pyridine ring protons exhibit large broadening in both cases. The oxidized cofactor has been examined in the same way, and here a series of dehydrogenases induce downfield shifts in everything. These binding-induced shifts, moreover, are sensitive to temperature and diminish in magnitude as the temperature is raised, that is to say as the conformational equilibrium in the free cofactor is progressively displaced in favour of the unstacked, open form. This indicates that it is the open form which corresponds to the state of the molecule when bound. NADH is more strongly bound than NAD⁺, so that the sticking time of the ligand is large, and only small shifts and large broadening are observed. The reason for the small perturbation of the adenine proton resonances is the absence of ring currents in the nonaromatic dihydropyridine ring. The observed shifts are pre-

Adhesiveness of T and B Cells

THE notion that T cells (of thymic origin) and B cells (derived from the bursa of Fabricius or its mammalian equivalent) can cooperate in the course of humoral antibody production is widely accepted. Quite plausible mechanisms have been advanced to explain how everything works—in *vitro* that is. Furthermore, it is reasonable to suppose that the various analytical experiments *in vitro* are not complete artefacts and that they give results which relate somehow to real life. But the technical difficulties in the way of ascertaining for sure what happens in the lymphoid system of an intact animal are formidable and many cellular immunologists have shied away from them. Curtis and de Sousa, however, more resolutely in *Nature New Biology* next Wednesday (July 11) present evidence which perhaps gets a little further to understanding the many factors likely to be involved in cooperation between T and B lymphocytes.

Curtis and de Sousa measure the adhesiveness of T cells, derived from the thymus by teasing, or B cells from mice which have previously been treated with antilymphocyte antiserum to remove some of the T cells, and artificial mixtures of such T and B cells. They find that each cell type has a characteristic adhesiveness with its own kind but that when the two sorts of cell are mixed a marked drop in overall adhesiveness occurs. Curtis and de Sousa favour the explanation that some soluble product of one or other or both of the two cell species affects the surface properties of

the cells in the mixture in such a manner as to facilitate their dispersion. This phenomenon they liken imaginatively to a similar drop in adhesiveness following mixing of cells from different strains of the sponge *Ephydatia fluviatilis*.

Curtis and de Sousa go on to show that supernatants from cultures of T cells reduce the adhesiveness of B cells in culture and *vice versa*. They also find that cells from unstimulated mouse lymph nodes have low adhesiveness—a result predictable from their artificial mixtures. Furthermore, cells from lymph nodes draining the site of application of the skin-sensitizing agent oxazolone have even lower adhesiveness. It would be fascinating if the authors were to apply their methods to other kinds of reacting lymph nodes in which the phenomenon of interaction between lymphocytes is better established—the response to sheep red cells is less spectacular than that to oxazolone but it is better analysed in terms of cooperating cells.

The authors feel that they may have stumbled upon a phenomenon by which systematization of cells during an immune response can be brought about. The results should be of interest to those who harvest efferent lymph in order to study reactive changes during a response to antigenic stimulus. Aside, however, from this, the communication perhaps marks the beginning of a swing back to a more physiological mode of investigation of the lymphoid system, away from the resolutely academic approach of immunologists.

sumably determined by interactions with side chains in the protein. In one unrelated dehydrogenase, different perturbations were observed, and Lee *et al.* suggest that the cofactor may be differently bound.

To come by definitive structures for nucleotide cofactors of lactate dehydrogenase, Rossmann and his colleagues (Chandrasekhar *et al.*, *J. mol. Biol.*, **76**, 503; 1973) have applied the constraints on dihedral angles that have been deduced from surveys of nucleotide and polynucleotide X ray structures. By this means the NAD structure in the ternary complex with pyruvate has been extracted and compared with bound NAD⁺ in the binary complex and with adenosine and its mono- and diphosphates. The precise geometry of the phosphate groups is evidently critical in determining the binding, and the induced conformational change in the protein, involving the movement of a loop of the polypeptide chain across the substrate cavity, can evidently be touched off by a change in phosphate ionization, for crystals of the apoenzyme disintegrate if the nucleotides are allowed to enter at a pH below the phosphate pK. Because adenosine mono- and diphosphates, when they bind at the cofactor site, elicit the same conformational switch as does NADH or NAD⁺, a direct comparison of their structure *in situ* is possible. The nucleotide structures are of the standard form, with the anti-configuration about the glycosidic bond, and a C_{3'}-endo pucker in the sugar, but small differences of detail are apparent between the different ligand and their environment, in the protein. The second phosphate of ADP is known not to contribute appreciably to the interaction energy, which fits with the new observation of two alternative, partly occupied, locations for this group. Shifts of up to 2 Å or so between phosphate groups in the different ligands can be seen.

Cognoscenti of dehydrogenases will also wish to give close attention to the accompanying paper by Adams, Liljas and Rossmann (*ibid.*, 519), who have compared the lactate dehydrogenase structure in crystals grown in sulphate and in citrate solutions. Two specific anion binding sites are present, one of them in the active centre cleft, and the interactions between the ionized and protonated carboxyl groups of the citrate ion with its surroundings in the protein have been defined. The second site is at a subunit interface, separated from the active centre by two arginine side chains, projecting from either side of an α helix. Adams *et al.* tentatively link this secondary anion binding site with a second substrate binding site, and with a stabilizing effect on the tetramer. Since citrate is known to inhibit the breakdown of the protein to

dimers, the site identifies the presumptive interfaces for this dissociation. The stable dimer unit is also thereby defined, and is structurally analogous to the dimeric malate dehydrogenase molecule (Rossmann *et al.*, *ibid.*, 533).

FISH BIOLOGY

Well Equipped Inio

from our Marine Vertebrate Correspondent
FISHES of the deep-sea genus *Benthalbella* are poorly known in general even though they are found in all tropical and warm temperate oceans. Their biology has been little studied beyond general observations concerning their bathymetric range; even their systematic relationships are not clear.

A study on *Benthalbella infans* by Merrett, Badcock and Herring (*J. Zool., Lond.*, **170**, 1; 1973) goes a long way towards rectifying the deficiencies in knowledge and clarifies an outstanding taxonomic problem. *Benthalbella infans* was described in 1911 by Zugmayer from specimens collected by the Prince of Monaco's yacht Princess Alice; this original specimen was small as its name suggests, and Zugmayer realized it was a juvenile. Relatively few specimens have been reported since, but in 1955 a second species, *B. dubius*, was described from a larger specimen and at the time it was suggested that this might be the adult of *B. infans*. Merrett and

his colleagues now report on ninety-seven specimens of *Benthalbella* caught by RRS Discovery over recent years. These new specimens nicely span the length range between Zugmayer's post-larval *infans* and the adult specimen of *dubius*; they show that the latter is indeed the adult *B. infans*.

The Discovery series of specimens provides much basic information on the developmental stages of *Benthalbella*, of which the changes in the eye are of great interest. The eyes are essentially tubular, except that the early post-larvae have lateral eyes which attain the adult condition first by a dorsal movement of the lens in respect of the eye cup, and as the eye grows a rotation, so that it is dorsally directed. This leaves the eyes as tubular organs forever looking upwards.

Also during development the body posture changes considerably. Young fish are hump-backed in the region of the forebody, but adults are straight-bodied or are even hollow-backed so that the top of the head is at a higher level than the midpoint of the back. Furthermore, the anterior vertebrae are unossified so that the head can be presumed to be unusually mobile in a vertical plane. Merrett *et al.* associate these structures and modifications with the deduced feeding behaviour of *Benthalbella*. Tubular, upwardly-directed eyes are presumed to be associated with detecting downcoming light from lumi-

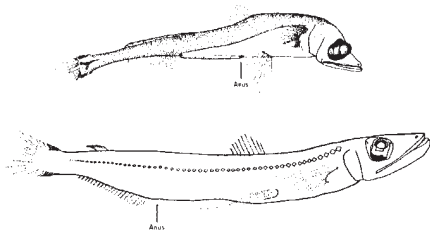
Sex Hormone Binding Proteins in Tissues

TARGET tissues for the sex hormones contain specific proteins which by interacting with the hormone are responsible for its uptake by the tissue and which are also involved in the early actions of the hormone. Binding proteins exist both in the nucleus and in the cytoplasm. In the case of the uterus the nuclear-binding protein has a sedimentation coefficient on ultracentrifugation of 4.5S, a molecular weight of about 60,000 and is relatively unstable, particularly in the absence of oestradiol. The cytoplasmic-binding protein is much larger (molecular weight of about 240,000, sedimentation coefficient 8.6S). The cytoplasmic protein can be broken down by exposure to buffers of high ionic strength to a smaller protein which in turn can be converted into the nuclear-binding protein by a factor present in the cytoplasm. Purification of the binding proteins has been difficult not only because of their low concentration in tissues but in the case of the nuclear-binding protein because of its instability.

Only poor yields and small degrees of purification have been achieved by conventional procedures or by attempts to purify the binding proteins chromatographically using columns containing oestrogens or oestrogen derivatives.

Recent developments in affinity chromatography have been more successful. In *Nature New Biology* next Wednesday (July 11) Sica *et al.* describe the purification of the oestradiol-binding protein from calf uteri by affinity chromatography using an adsorbent oestradiol-17-hemisuccinate coupled by the carbodiimide procedure to an agarose derivative. In the purification of material present in such low concentration special care must be taken to remove non-covalently-bound oestradiol from the solid phase before chromatography and the methods must be sufficiently sensitive to detect the elution of the material.

The oestrogen-binding protein eluted from the adsorbent had similar physical characteristics to that of the nuclear receptor. The factors involved in the selective absorption and elution of the receptor proteins are also discussed in some detail. Although in these particular experiments the degree of purification achieved was not large, the yield obtained in most experiments was satisfactory. The authors suggest that by modification of the adsorbent more than a 10,000-fold purification of the receptor protein can be obtained but would still be only 20–40% the purity.



Post-larva (56.0 mm standard length) (above) and adult stage (124.0 mm standard length) (below) of *Benthallia infans* (from Merrett *et al.*, *J. Zool., Lond.*, **170**, 1; 1973).

nescent organisms, or the silhouettes of prey against the lighted sea surface. It is suggested that during active feeding prey is stalked from below and struck at by a rapid backward arching of the forepart of the body. The head is assumed to be capable of an extra swing on account of the flexibility of the anterior vertebrae, thus widening the gape. The jaws are well equipped with teeth and *Benthallia* is evidently capable of catching large prey as an adult, even if it does seize its prey by this half back-spring method.

Adult *Benthallia* have also been found to have four ventral light organs which are lemon yellow in colour, but are not clearly visible, for the melanophores which lie in the epidermis covering them almost occlude the organ when expanded. This ability to mask the ventral lights could clearly be an advantage. The formation of these light organs is of a type rarely observed in deep-sea fishes, for they seem to be derived from muscular tissue and their luminescence is presumably controlled by modified muscle innervation. These light organs do not become fully developed until maturity and Merrett *et al.* suggest that their main function is specific recognition between sexually mature animals, the downward-pointing lights and upward-looking eyes being advantageous in a species with apparently solitary habits.

The discovery that *Benthallia*, like other inious fishes, is hermaphrodite, with simultaneously maturing ovarian and testicular tissue, is perhaps only to be expected in this interesting fish which is so well equipped for productive encounters between specimens and for life in the deep sea.

GENETICS

Mutagenic Effects

from a Correspondent

THE third meeting of the European Environmental Mutagen Society took place in Uppsala on June 4–7 and was opened by Dr B. A. Bridges (MRC Cell Mutation Unit, Brighton) who suggested some general principles of mutagenicity

screening and proposed a three-tier framework for evaluating genetic hazards of new compounds. Compounds would be assayed qualitatively for mutagenicity by *in vitro* tests if consumed in small quantities (tier 1), or *in vivo* in animal systems if consumed in large quantities (tier 2). Food additives, medicines and some pesticides would fall into the second category. Substances found to be mutagenic by these tests, but considered to be of great value and having no known substitutes, would then be subjected to a quantitative risk/benefit analysis (tier 3).

Some of the difficulties likely to be encountered in such a thorough screening procedure became apparent in a contribution by Dr F. J. de Serres (National Institute of Environmental Health Sciences, NIH, Bethesda) who described a case study on hycanthone, a drug used by the World Health Organization for mass chemotherapy of schistosomiasis in the tropics. Hycanthone was found to be mutagenic, teratogenic and carcinogenic in a variety of systems. Attempts to find structural analogues which retain the desirable pharmacological properties but are free of undesirable toxicological properties, however, led to the discovery that the analogues were in some cases more, in

others less, mutagenic than hycanthone itself.

Dr J. A. Miller (University of Wisconsin) discussed the relationship between mutagenicity and carcinogenicity. The active forms of carcinogens and mutagens are both electrophilic reactants, suggesting a strong formal relationship between the two processes, even though they may not be causally related. Hence many mutagens are likely to be potential carcinogens. Because most chemical carcinogens require metabolic activation, Dr Miller stressed the importance of including activation enzymes in mutagenicity test systems for detecting potential carcinogens. One way of carrying this out is by using host-mediated assays. An alternative new method, developed by Dr B. Ames, whereby the mutation test system includes a rat liver microsomal fraction to activate the potential mutagen as a substitute for the host-mediated assay, seems very promising. Such a rat liver system has also been used with success by Dr R. Barale (Institute of Genetics, Pisa) who included it in an assay to measure the production of gene conversions in yeast by ethyl methane sulphonate. Dr P. Brookes (Chester Beatty Research Institute, Pollards Wood) discussed the mechanism of the mutagenic

β_2 Microglobulin Synthesis and IgG Production

ONE of the most esoteric of biological time perspectives derives from studies of molecular homologies. Histone proteins, for example, which are arguably the most ubiquitous proteins, are nearly completely identical in all species of plants and animals that have been studied. This probably means that the genes which code for the histones have remained largely unchanged since the parting of the plant and animal kingdoms hundreds of millions of years ago.

In *Nature New Biology* next Wednesday (July 11), Nilsson and his colleagues present another example of delving into the past by studying β_2 microglobulin which is a low molecular weight protein found in human biological fluids and, apparently, the urine of dogs. They point out that this protein is homologous with the constant domains of the light and heavy chains of immunoglobulin (IgG) and has, in addition, an intrachain disulphide loop of the same size and location as those found in the constant domain of IgG.

Nilsson *et al.* sought β_2 microglobulin on some twenty-nine different tissue culture cell lines—some lymphoid, others non-lymphoid. In carrying out this exercise they were trying to ascertain whether β_2 microglobulin was only to be found in association with cells of the leukocyte series and whether its synthesis could be in any way linked with the synthesis of IgG.

All the non-lymphoid cell lines produced considerable amounts of β_2 microglobulin. The lymphoid cells were usually more variable in the amount produced, one out of eight being consistently negative. Some lymphoma lines which did not produce soluble IgG nevertheless synthesized β_2 microglobulin. Nilsson *et al.* go to considerable trouble to show that the β_2 microglobulin produced *in vitro* is identical with that found in serum and urine.

Their overall conclusion is that there is no correlation between the capacity to synthesize immunoglobulin and the production of β_2 microglobulin. And yet the sequence similarity between IgG and β_2 microglobulin seems to point to an ancestral common gene. The four constant domains of the IgG are thought to demonstrate gene duplication and Nilsson *et al.* suppose that a similar duplication led to the now independent faculty for β_2 microglobulin synthesis. There is now more understanding of the biological significance of β_2 microglobulin, but this elegant demonstration that the regulation of its production is now divorced from the production of IgG promotes the biological time-traveller to wonder when the divorce occurred. Perhaps clues will emerge from a study of the sera of living representatives of the primitive vertebrates.

effects of some carcinogens in microbial and mammalian cellular systems.

Dr W. Nichols (Institute of Genetics, Lund) discussed the use of chromosomal aberrations as an indicator for gene mutations. There is a qualitative correlation between the ability of agents to produce chromosome aberrations and gene mutations, but quantitatively the correlation is not good. Furthermore, there is no indication that the mechanism of production of chromosome breaks and mutations is the same. The very great advantage of measuring chromosome aberrations, however, is that it is at present the only available method of detecting effects in man.

One day of the meeting was devoted to a symposium on the effects of caffeine as an environmental mutagen. Dr B. A. Kihlman (Institute of Genetics and Plant Breeding, Uppsala) reviewed many of the known effects of caffeine, describing in particular the work of his own and other groups on the production of chromosome breaks by caffeine in plant and animal cells, and the very powerful potentiating effect of caffeine for cell killing and production of chromosome breaks by other agents (for example, ultraviolet light, alkylating agents).

Dr A. R. Lehmann (University of Sussex) showed that, biochemically, caffeine specifically inhibits the filling in of daughter-strand gaps opposite lesions in damaged DNA, thus linking effects at the molecular level to observations at the chromosomal and cellular level. Dr J. J. Roberts (Chester Beatty Research Institute, Pollards Wood) showed that inhibition of this post-replication repair process could lead to chromosome damage, mutagenesis and cell death. Dr F. H. Sobels (University of Leiden) described work by Dr D. Mendelson and himself, using mutant strains of *Drosophila*. Caffeine appeared to inhibit a system in female flies which after mating repaired damage produced in the sperm of X-irradiated males. There was general agreement, however, that at the plasma levels produced by consumption of common beverages, caffeine is probably not a hazard, and there certainly was no apparent curtailment of coffee consumption after the symposium.

In the contributed papers mutagenic effects of, for example, various chemicals and pesticides in a wide variety of systems were reported. The test systems ranged from simple microorganisms through host-mediated assays to measurements of dominant lethals, specific locus changes in mice and chromosome aberrations in man. Several groups presented results with eukaryotic microorganisms as the test system, and they suggested that simple experiments with nucleated organisms are likely to be of more relevance to man than are corresponding experiments with prokaryotes.

SELENOLOGY

Stepwise Velocity

from our Geomagnetism Correspondent

By any standards, the most important and rewarding lunar "geophysical" data to emerge from the Apollo project must surely be those derived from the various seismological experiments. As a result of pre-Apollo 17 studies, the P wave velocity in the Moon is now known to increase rapidly from 0.1–0.3 km s⁻¹ in the upper 100 m to about 6 km s⁻¹ at depths of 15–20 km. But because of the lack of seismic travel time data at source-detector distances of less than 30 km, the details of this overall variation have remained obscure and the structure of the upper 10 km of the Moon is thus poorly defined. In the absence of information to the contrary, some workers have therefore assumed the velocity-depth distribution for P waves to be smooth. Kovach and Watkins (*Moon*, in the press), for example, have taken this line, but also suggest that a rate of velocity increase of about 2 km s⁻¹ per kilometre could hardly be explained solely by the effect of increasing pressure.

But such is the present rate of progress in lunar research that Kovach and Watkins (*Science*, **180**, 1063; 1973) are now able to report that their own previous beliefs have been overtaken by events. During the Apollo 17 mission

a triangular array of four seismometers was successfully installed in the Taurus-Littrow region of the Moon and recorded strong seismic signals from eight explosive charges and the crash of the lunar module ascent stage. The charges gave seismic travel time data in the distance range 0.1–2.7 km only, but the source-detector distance was increased significantly by the impact of the lunar module 8.7 km away.

The resulting time-distance graph clearly shows that, in the Taurus-Littrow area at least, the Moon is layered, for the P wave velocity increases with depth in a stepwise manner. Because the minimum source-detector distance achieved was about 100 m, the structure of the upper 20 m or so could not be resolved. Below this thin surface shell, however, the measured seismic velocity is 0.25 km s⁻¹ over a depth range of 248 m, rising abruptly to 1.2 km s⁻¹ for a further 925 m. The velocity then increases sharply to 4 km s⁻¹, although because of the paucity of data at distances greater than 3 km from the array (the only point is that from the lunar module impact) there is some uncertainty in this value. But precise values apart, the important point is that material with velocity ≥ 4 km s⁻¹ underlies the 1.2 km s⁻¹ material.

So much for the physical structures of the upper few kilometres of the Moon. But is it also possible to deduce anything about the nature of the seismic

Precambrian on the Rockall Bank

In *Nature Physical Science* next Monday (July 9), Roberts, Ardu and Dearnley report on material obtained from drilling into two solid outcrops sixty miles apart on the Rockall Bank. At both sites they obtained granulite facies metamorphic rocks and so confirm conclusively what had been deduced from earlier work. At a site on Briony Bank they found an acid granulite containing a relatively small amount of orthopyroxene and even less clinopyroxene. The petrography is similar to that of hypersthene granulite dredged from the vicinity of Rockall Bank. At a second site they found an orthopyroxene-bearing granulite. Both rocks could readily be matched in the Outer Hebrides and north-west Highlands. The whole rock date of $1,670 \pm 24$ m.y. and that on hornblende at $1,566 \pm 33$ m.y. indicate a Laxfordian age for the material, although the authors point out that still older Scourian rock may be present.

The demonstration of Roberts *et al.* that part of Rockall Bank is underlain by such Precambrian rocks is of considerable geological interest. In southern Greenland granulite facies rocks between 1,800 and 1,600 m.y. old are present and in both Greenland and north-west Scotland considerably older

granulite facies rocks approximately 3×10^9 yr old are widespread. Furthermore, pebbles in the younger Late Precambrian Torridonian formation of north-west Scotland known to be derived from the west of the British Isles include high grade metamorphic rocks in these two age groups. It would seem that the way is now open for a more precise reconstruction of the Precambrian rocks of north-west Europe, of the microcontinent below the Rockall Bank and of Greenland.

The boundary of the Precambrian Grenville province, as Roberts, Ardu and Dearnley point out, may lie at the southern edge of the Rockall Bank. This province lies to the south of Greenland and has never been recognized in northern Scotland, though rocks of Grenville age occur outside Canada, in southern Norway and the Western Approaches to the English Channel. The new work could lead to the location of this important structure which apparently crosses the British Isles, dividing the basement below Britain into an older north-westerly section of Laxfordian and Scourian and Prescourian age ($> 1.4 \times 10^9$ yr) and a younger south-easterly position formed since 1.2×10^9 yr ago.

velocity zones? Much of the Taurus-Littrow site area is apparently covered by a dark mantling material, and presumably at some depth this gives way to the subfloor material represented by the velocity of 0.25 km s^{-1} . Because of the lack of resolution in the upper 20 m, Kovach and Watkins have been unable to discover whether this boundary is sharp or gradational; but whether or not there is a well-defined interface, the change from mantling material to subfloor must clearly take place in the upper 20 m.

Geological examination of the subfloor material itself (for example, where it is exposed in crater walls) shows it to be a medium-grained vesicular basalt with textural variations implying the presence of individual flows. Kovach and Watkins interpret both the 0.25 km s^{-1} material and the 1.2 km s^{-1} material as basalt flows; and certainly these velocities fall within the range of terrestrial basalts. On the other hand, the abrupt change from one velocity to the other implies a major change in the formation or evolution of the subfloor basalts. One possibility is that the lower velocity flow or flows are fractured or brecciated, and, again, there is a terrestrial precedent for this. In any event, the combined thickness of 1,173 m appears to account for all the subfloor basalts at the Taurus-Littrow site.

Finally, as only to be expected, the nature of the underlying $4+ \text{ km s}^{-1}$ material is more difficult to define. The most likely candidate seems to be the coherent breccia which dominates in the surrounding highland massif. Insofar as this breccia appears to be similar to that obtained at the Apennine front by Apollo 15 and in the Descartes region by Apollo 16, support for its underlying the basalt comes from laboratory studies in which Todd *et al.* (*Geochim. Cosmochim. Acta*, **3**, 2577; 1972) measured comparable velocities.

SEMICONDUCTORS

CCD Prospects

from a Correspondent

CHARGE coupled devices (CCDs) have attracted much attention since their development at Bell Telephone Laboratories in 1970, because of their potential as fast semiconductor memories and imaging devices with high packing densities and low power consumption, and the fact that, at least in concept, they are simple to fabricate. The importance now attached to these devices by the electronics industry was reflected by the attendance at an Institute of Physics meeting at Imperial College, London, on June 11. It is somewhat unfair to say that all participants were interested solely in charge coupled devices as the meeting was officially titled

"Charge Transfer Devices" and three out of the eight contributions discussed MOS and bipolar bucket brigade devices which offer advantages in some particular applications, such as correlators and filters. Existing bipolar devices operate at clock rates of more than 10 MHz and have storage times longer than 10 ms.

In a CCD minority carriers, which are stored at the surface of a semiconductor in potential wells, can be transported along that surface by means of suitable potentials applied to an array of closely spaced metal electrodes separated from the semiconductor by an insulating layer.

After the introductory papers on both CCDs (Mr D. J. Burt (GEC)) and bucket brigade devices (Mr P. W. Rivers-Latham (Plessey)) there was a series of contributions from GEC which described the fabrication and performance of different CCDs for both shift registers and imaging devices. These ranged from two-phase two-level electrode and implanted barrier devices, with poten-

tial packing densities of 10^5 bits per package for serial memory applications, through 100 element three-phase single level electrode devices for analogue delay lines with maximum delays of 4 ms and 10 kHz bandwidths at low frequencies (or $25 \mu\text{s}$ delays and 1 to 2 MHz bandwidth at 4 MHz clock rates) to optical imaging devices with up to 100×50 element area imaging arrays plus a 100×49 element information store and a 100 element linear readout, all on the same chip.

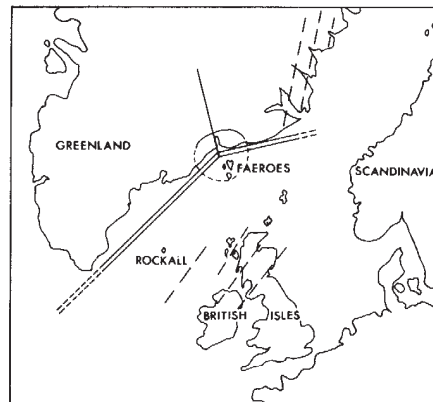
The picture painted was an optimistic one of the future of CCDs but it was clear that there are problems in obtaining sufficiently high charge transfer efficiencies to make more than 100 element linear arrays without the use of on-chip charge regenerators. Figures quoted for charge loss per transfer ranged from $3 \times 10^{-3}\%$ to $3 \times 10^{-2}\%$ with the use of "fat zeroes", although it was clear in subsequent contributions from the Royal Radar Establishment and the University of Southampton that more work is required on both the

Early Effects of Icelandic Plume

GILLULY (*Bull. geol. Soc. Am.*, **82**, 2383; 1971) recently concluded that it is difficult to relate the large volume of plateau basalt lavas in East Greenland to "any mechanism of plate tectonics". In *Nature Physical Science* next Monday (July 9), however, Brooks apparently overcomes this difficulty by proposing that the East Greenland flood basalts are the result of what some people would now regard as the most fundamental of all plate tectonic mechanisms—the mantle plume. In particular, he suggests that the basalts, and associated doming and rifting, represent the early stages of the activity of the supposed Icelandic mantle plume, thus following Gass (*Phil. Trans. R. Soc.*, **A267**, 369; 1970) who first applied the basic idea to crustal doming in the Red Sea and East African regions.

The particular dome investigated by Brooks lies in the Kangerdlugssuaq area of East Greenland and probably formed just after, or partly during, the basalt production which marked the advent of the Icelandic plume. In plan, the dome is now roughly elliptical in shape with a long axis of several hundred kilometres, and lies along the Greenland coast (see map). But according to Brooks's interpretation, it would originally have been more circular, extending out into what is now ocean and including the Faeroes in their pre-drift position. During its early history, the dome was then subject to Y-shaped rifting, the two rift arms lying along the East Greenland coast being active and thus giving rise to lithospheric spreading and the creation of new ocean floor. The third arm, striking inland, was inactive.

The inactive arm, now represented by a wide fjord, has some of the characteristics of continental rift valleys. The magmatic rocks along it, for example, become progressively more alkaline away from the triple junction. By contrast, the two spreading rifts are characterized by "plume-type tholeiites". In addition, the coastal flanks of the present dome are associated with an intense dyke swarm within which the basement gneisses are present only as very thin sections. A dyke swarm of this density is reminiscent of the type of dyke production responsible for the creation of oceanic lithosphere and suggests parallels with the Troodos (Cyprus) sheeted diabase complex, also thought to represent the result of ancient ocean floor spreading. Brooks thus believes that the coastal area around Kangerdlugssuaq preserves another segment of old ocean floor.



The pre-drift configuration of the NE Atlantic showing the extent of the dome postulated by Brooks and the triple junction formed by dome rifting.

techniques of measuring charge transfer efficiencies and on the effects of surface states at the semiconductor surface on these transfer efficiencies. The latter problem has in fact led to the development of the so-called peristaltic CCDs. Dr L. J. M. Esser (Philips) described how the charge packets in these devices (majority carriers rather than minority carriers) are held away from the semiconductor surface in drift fields which are an order of magnitude higher than those in conventional CCDs. This has led to experimental four-phase devices with charge losses per transfer of less than $10^{-4}\%$ at clock frequencies of more than 100 MHz and to the prospects of operation at microwave frequencies.

The meeting was brought to a close by the chairman, Dr F. H. Reynolds (Post Office Research Station, Dollis Hill), who pointed out the lack of discussion at the meeting of the technology required to make CCDs. This, he suggested, indicates that people are confident that the present difficulties encountered in, for example, defining several centimetres of inter-electrode gaps only $2\text{ }\mu\text{m}$ wide in the 100×100 arrays would be overcome and that large area arrays would become available in the relatively near future at prices much below the present level of around £1 per bit. Nobody was prepared to commit themselves to saying that a particular technology or particular clocking system was the "best", although it does seem likely that at present the most promising applications of CCDs are in the fields of imaging.

PARTICLE DETECTORS

Wiping Clean

CHARGED particle tracks can now be recorded, and erased at will, with a specially modified crystal of strontium titanate. All that has to be done to remove any trace of, for example, an alpha particle is to heat the crystal to 900°C in a partial vacuum. This is reported by Ouseph in a recent issue of *Physical Review Letters* (30, 1162; 1973).

To manufacture one of these crystals about 0.1% of the titanium ions in a normal crystal of strontium titanate have to be replaced with iron ions, about half of which are Fe^{2+} and half Fe^{3+} . Such a crystal is black because Fe^{2+} ions cut out visible light.

If, however, the balance is disturbed in favour of Fe^{3+} the colour of the crystal changes to yellow because the physical properties of the Fe^{3+} ions predominate. In these circumstances there is a movement of negative oxygen ions to maintain charge balance in the crystal as a whole. In fact a higher than average concentration of Fe^{2+} ions anywhere in the crystal is accompanied by a higher than average concentration of oxygen ions.

If a doped crystal of strontium titanate, about 2 cm across and 2 mm thick, is sandwiched between two metal electrodes and a potential difference of 1,000 V applied across it when the temperature is greater than 100°C , negative oxygen ions are attracted to the positive electrode and the vicinity of that electrode becomes black because Fe^{2+} ions also accumulate there.

Because a charged particle passing through this arrangement at room temperature can induce local heating as it slows down and loses energy, blackening will occur on the side of the track to which oxygen ions are attracted by the electric field applied. Unfortunately it is not possible to pick up individual particle tracks with an optical microscope because they are too small, but blackening has been observed by Ouseph by exposing the crystal to a beam of alpha particles from a particle accelerator.

Tracks of single particles can, however, be detected satisfactorily if a thin layer of uranium oxide (about 0.1 mm) is put in between the positive electrode and the strontium titanate crystal. Ouseph detected individual alpha particles given off radioactively by the uranium in this layer and he explains

this in terms of the release of positive oxygen ions by the alpha particles as they slow down in the oxide layer. These ions are then attracted to the surface of the strontium titanate where Fe^{2+} ions also congregate, and black spots appear, one per alpha particle, which can be seen with a microscope.

Ouseph has also detected a cosmic-ray particle which travelled lengthways through the oxide layer, approximately parallel to the electrode and the surface of the crystal, releasing oxygen ions as it went and eventually reacting with a nucleus in the same layer.

All these tracks can easily be erased by heating up the crystal to 900°C in a partial vacuum, so the device has obvious advantages over photographic emulsions which are widely used by cosmic-ray physicists to detect charged particles but which cannot be recycled.

Ouseph also points out that the new method, although at an early stage of development, evidently has the same advantages over particle detectors like cloud chambers and bubble chambers as do photographic emulsions—it is continuously sensitive and does not have to be triggered when it is required to record the passage of particles.

Worldwide Marine Sedimentary Hiatuses

A HIATUS in a sedimentary column, a well known geological phenomenon in both marine and continental environments, may be recognized by a discontinuity in palaeontologically determined ages, an abrupt and distinct decrease in sedimentation rate, or both. Moreover, where sedimentation ceases completely for a period during which erosion may take place, a surface of unconformity may form to mark the hiatus more clearly. But as far as physical causes are concerned, the hiatus is, in a sense, asymmetrical. The change from sedimentation to non-deposition or erosion may represent the reaching of an erosional level which does not necessarily correspond to the beginning of an associated physical event. By contrast, the end of a hiatus must mark the end of the physical event.

In *Nature Physical Science* next Monday (July 9), Rona presents an analysis of marine sedimentary hiatuses in terms of physical causes. The Deep Sea Drilling Project (DSDP) has revealed the presence of hiatuses up to tens of millions of years long in all the principal ocean basins, and in the Atlantic, Pacific and Indian Oceans and the Caribbean Sea hiatuses are recognized at 51 out of 171 sites. Rona shows that two of the hiatuses are apparently synchronous throughout the principal basins. The first of these involves more than half of the Palaeocene and ends during the Palaeocene or early Eocene; the second occupies more than half of

the Oligocene and ends during the early to middle Miocene.

Because these two hiatuses are worldwide, their endings must mark the completion of physical events of worldwide extent. Rona suggests that these physical events are eustatic changes of sea level. The Palaeocene hiatus corresponds to a worldwide reversal from epicontinental marine regression to transgression, and the Oligocene hiatus correlates with a worldwide regression. Such eustatic changes would be expected to lead to "interacting physical and chemical effects on a global scale" and thus to sedimentary hiatuses on a world scale.

The chief physical effect is likely to be changes in ocean circulation leading to erosion by bottom currents. This is supported by the fact that the hiatuses generally lasted longer in the western Atlantic and Pacific basins than in the eastern basins where weaker deep circulation has been predicted. Evidence for associated chemical changes comes from the relatively high average carbonate content of the Palaeocene-early Eocene Atlantic basin sediments and of Oligocene sediments from both the Atlantic and the Pacific. On the other hand, Rona points out that during the Palaeocene and Oligocene the transfer of sediment from continent to ocean basin would have been relatively large. Thus in spite of the hiatuses, there was a net accumulation of sediment in the ocean basins.

Fossil Occurrences at Langebaanweg, Cape Province

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The phosphate quarries at Langebaanweg are yielding prolific and important assemblages of late Cainozoic fossils.

FIFTEEN years have elapsed since fossils were first recorded from the phosphate quarries at Langebaanweg, 104 km north-north-west of Cape Town¹. Since then the occurrences have become one of the most prolific sources of late Cainozoic fossil vertebrates and invertebrates in southern Africa and they have yielded the most important assemblage of Pliocene fossils yet recorded from this region.

During 1968 the South African Museum formulated a research programme aimed at the systematic recovery and investigation of fossils from the site of present mining operations on the farm Varswater (E Quarry, Fig. 1) and four field seasons of one to two months each winter have since been completed. The surface collection and excavation of fossils has been largely confined to the floor of the quarry, an area of approximately 170,000 m² which will soon be reburied as the quarry is to be back filled. Highly fossiliferous deposits of the west wall of the quarry are not scheduled to be mined for many years and offer a potential for future palaeontological exploitation.

Most of the fossils occur in unconsolidated gravel and sand and a method of recovery which is often used is to wash the deposit through sieves of varying mesh-size, a process which has led to the discovery of fossils as small as isolated teeth of insectivores and rodents. The bones of larger animals exposed at the surface are often damaged during mining operations and consequently much time has been devoted to controlled excavations into *in situ* deposits. The recovery of the remains of large species by this process is largely fortuitous, but smaller species are more abundantly represented and are found in greater quantities.

An investigation into the geology of E quarry and the surrounding areas is being undertaken in conjunction with the fossil recovery programme and the deposits are now categorized as follows.

Surface bed. This unit is made up of essentially unfossiliferous, Pleistocene aeolian sands varying in depth from 2 to 40 m and incorporating calcretes and palaeosols.

Varswater Formation. This comprises the phosphatic and fossiliferous deposits exposed in E quarry. Four principal units are recognized. (a) Bed 3b: medium to coarse-grade phosphatic sand from which very few fossils have been recovered. (b) Bed 3a: medium-grade phosphatic sand exposed only on the west wall of E quarry which includes numerous vertebrate fossils belonging chiefly to terrestrial species. (c) Bed 2: medium-grade and largely non-phosphatic sand exposed over most of the floor of the quarry and which includes numerous fossils belonging principally to terrestrial species. (d) Bed 1: a beach gravel made up of phosphate rock pebbles, cobbles and boulders in a sand matrix and in which marine vertebrate and invertebrate fossils are predominant.

Basal clay. The whole sequence is underlain by a clay of unknown origin and unknown depth.

The dating of deposits in the Langebaanweg area has come to depend on a correlation of the fossil mammal faunas with securely dated faunas from East Africa^{2,3}. An age of about 4 m.y. for the Varswater Formation has been inferred, although a still uncompleted study of the pig, *Nyanzachoerus*, suggests that the actual age may be nearer 4.5 m.y. The E quarry

Nyanzachoerus is apparently not conspecific with previously recorded species from East and North Africa. It is more advanced than *N. syrticus* from Sahabi⁴ and *N. tulotis* from Lothagam⁵, but is less advanced than later species from Kanapoi⁵, Kanam⁶, Hamada Damous⁷ and elsewhere. If a date of 4.5 m.y. for the E quarry fauna is correct, then the East African faunas most similar to it in age are probably those from the Mursi Formation in the Omo Valley and Kubi Algi east of Lake Rudolf⁸.

The fauna from the mined-out Baard's quarry includes a Pleistocene element³, but the available fossils from this occurrence are limited in both quantity and quality, and the fauna has little palaeontological significance.

Fauna of Varswater Formation

The E quarry fauna is unique among those from important late Cainozoic fossil occurrences in sub-Saharan Africa in that it includes an association of material from marine, freshwater and terrestrial habitats. It is also particularly significant because little has previously been known of the Pliocene fauna of the subcontinent. The fossils are in general very well preserved and, although usually only isolated skeletal elements are recovered, partial skeletons are now being found with increasing frequency as a result of the controlled excavations. The material is recorded according to the bed from which it is recovered and, although beds 1, 2 and 3a do have species in common, there are also marked differences in the representation of species in the three stratigraphic units.

A preliminary study of the marine invertebrates from bed 1 was recently completed⁹, and about twenty molluscan species, a brachiopod, an acorn barnacle, and a sea urchin were recognized.

The remains of sharks, skates and rays, most of which are from bed 1, are currently being investigated. The rather limited bony fish material, however, is still unstudied, although both mussel-cracker and catfish are known to occur.

The most abundant fossils in E quarry are of tortoises which may occur even in the poorly fossiliferous parts of beds 1, 2 and 3a. Apparently only a single species of the genus *Chersina* is represented, although at least one incomplete skeleton from bed 2 may belong to a second species. Other reptiles include snakes and lizards, but crocodiles are a notable absentee from the fauna.

Bird remains are very common in certain areas and the species range in size from a very small to a very large ostrich. Water birds such as duck and cormorant are included in the remains and the francolin, plover and ibis. A penguin from bed 2, the first African fossil record of this order, was recently described¹⁰.



Fig. 1 E quarry, Langebaanweg, with bed 2 of the Varswater Formation exposed on the floor of the quarry and the mechanical excavators in the background excavating bed 3.

The mammals from E quarry have been the principal focus of attention (Table 1), although relatively few of the approximately sixty recorded species have yet been described. The exceptions include an elephant¹¹, a seal¹² and a rhinoceros¹³. The *Hipparion* from other occurrences in the Langebaanweg area has also been studied¹⁴, but not the material from E quarry. The diverse fissiped carnivore assemblage has been investigated, but only the description of the bear, *Agriotherium africanum*, has so far been published¹⁵.

A feature of the fauna is that it includes representatives of groups which are or were essentially Eurasian in their distribution (for example, *Agriotherium*, *Percrocuta*, a boselaphine), although groups which are exclusively or essentially "African" in character are well represented. The latter include an elephant shrew (Macroscelididae), a golden mole (Chrysochoridae), an armadillo (Tubulidentata) and giraffes (Giraffidae). *Hippopotamus*, like the crocodile, is unexpectedly absent.

One of the more remarkable records from E quarry is that of the monachine seal, *Prionodelphis capensis*. This is only the second record of the genus, the other being from the Argentine, and it provides the first good evidence of the nature of the geographical and morphological link between the monk

seals of the Northern Hemisphere and the seals of Antarctica¹⁶.

Thirteen of the fifteen mammalian orders which still occur in Africa and adjacent oceans are recorded from E quarry, the only exceptions being the bats and sirenians. Although so wide a variety of mammals and other vertebrates are present, the representation of individual species is very variable. Three mammalian orders, namely primates, pangolins and armadillos, are known only from a few isolated teeth and postcranial bones, and this renders the identification of the species concerned difficult, if not impossible. On the other hand, remains of some small mammals are very abundant, and larger mammals such as the rhinoceros¹³ are quite well represented.

Palaeoecology

It can now be concluded from the fossil and geological evidence available that the Varswater Formation was laid down during a marine transgression which peaked at about 50 m above present sea level. At that time a river, probably a precursor of the present Great Berg River, had its mouth in the vicinity of E quarry. This river was apparently an important factor in both attracting animal life to the area and subsequently affecting the dispersal of their remains. The latter role was evidently shared by carnivores because there is evidence both in the nature of damage to and dispersal of bones that predators and scavengers contributed to the dismemberment of carcasses. This activity probably took place in and adjacent to the river and its estuary, because both terrestrial and aquatic carnivores are represented in the assemblage.

There is to date no direct evidence concerning the nature of the vegetation in the Langebaanweg area in late Pliocene times, but the large and varied herbivore fauna suggests that it was more luxuriant than at present. The mean annual rainfall today is about 250 mm or less, it falls chiefly in winter and is sufficient to support only a Mediterranean macchia vegetation with no indigenous trees. The long-necked giraffe from E quarry indicates that trees were present during the late Pliocene, while the high-crowned teeth of both rhinoceros and horse suggest the presence of grasslands as well. The bovid which is most common in bed 2 is the boselaphine, which was apparently an open woodland species. The environment visualized is that of a riverine woodland flanked by grasslands.

Burnt bone in the deposits may result from bush fires similar to this which occur on the African savannas during the dry season. If there was a pronounced dry season in the area during the late Pliocene, then there might well have been a greater concentration of animal life in the vicinity of the "Langebaanweg River" at these times, so that much of the E quarry assemblage may represent dry season accumulations of animal remains on the floodplain of the river.

The field work at Langebaanweg is facilitated by Chemfos Limited, a subsidiary of the African Metals Corporation, and the Wenner-Gren Foundation for Anthropological Research. The undertaking is otherwise financed by the South African Museum and the South African Council for Scientific and Industrial Research.

Table 1 Mammalian Fauna of the Varswater Formation, E Quarry

	Bed 1	Bed 2	Bed 3a	?
Insectivora				
At least four species		×	×	
Primates				
(?) One species		×		
Pholidota				
One species		×		
Tubulidentata				
One species		×		
Carnivora				
Canidae gen. et sp. indet.			×	
<i>Agriotherium africanum</i>			×	
<i>Mellivora</i> aff. <i>punjabiensis</i>				×
<i>Enhyriodon africanus</i>				×
(?) Lutrinae gen. et sp. indet.		×		
<i>Viverra leakeyi</i>		×	×	
<i>Genetta</i> sp.		×		
<i>Herpestes</i> (two species)		×	×	
<i>Percrocuta</i> sp.		×		
<i>Hyaena</i> (? three species)		×	×	
<i>Hyaenictis</i> sp.			×	
<i>Machairodus</i> sp.		×		
<i>Machairodontinae</i> gen. et sp. indet.			×	
<i>Felis</i> aff. <i>issiodorensis</i>			×	
<i>Felis</i> sp. (aff. <i>Sivafelis</i>)			×	
<i>Dinofelis</i> aff. <i>diastemata</i>		×	×	
Pinnipedia				
<i>Prionodelphis capensis</i>	×	×	×	
Proboscidea				
Gomphotheriidae gen. et sp. indet.		×		
<i>Mammuthus subplanifrons</i>		×		
Hyracoidea				
aff. <i>Procavia antiqua</i>			×	
Perissodactyla				
<i>Ceratotherium praecox</i>		×		
<i>Hipparion albertense</i>	?	×	×	
Artiodactyla				
<i>Nyanzachoerus</i> sp.		×		
Suidae gen. et sp. indet.			×	
<i>Libytherium</i> sp.		×	?	
<i>Giraffa</i> cf. <i>gracilis</i>		×		
<i>Tragelaphus</i> sp.				×
Bovini gen. et sp. indet.				×
Boselaphini gen. et sp. indet.		×		
Reduncini gen. et sp. indet.				×
Alcelaphini (two species)		?	×	
Neotragini (aff. <i>Raphicerus</i>)		×		
<i>Gazella</i> aff. <i>vanhoepeni</i>				×
Bovidae gen. et sp. indet.		×		
Lagomorpha				
One species		×	×	
Rodentia				
Bathergidae (two species)		×	×	
Muridae and ? others (many species)		×	×	
Cetacea				
Several species	×			

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Control of Ribosomal RNA Synthesis *in vitro*

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The closed rRNA promoter and the protein synthesis elongation factor TuTs are prerequisites for the selective control of *in vitro* rRNA synthesis by ppGpp.

ribosomal RNA (rRNA) synthesis in bacteria is regulated independently of much mRNA synthesis¹⁻³. A putative negative effector of this regulation is ppGpp, a guanine nucleotide whose accumulation *in vivo* is correlated with the inhibition of rRNA synthesis^{4,5}. *In vitro* preferential inhibition of rRNA synthesis by ppGpp has been observed only when such synthesis is dependent on the transcription factor, ψ r^{6,7}, now equated with the protein synthesis elongation factor, TuTs⁷. I show here that enhancement of rRNA synthesis by TuTs is determined by the configuration of the DNA template and suggest that the factor alters the specificity of transcription by stabilizing a particular initiation conformation of RNA polymerase holoenzyme.

TuTs, a Transcription Specificity Factor

The *in vitro* synthesis of *Escherichia coli* rRNA is strongly dependent on both the conformation of the DNA template and the KCl concentration⁹. At 0.1 M KCl two forms of the rRNA promoter can be distinguished; below 35° C the promoter is closed, above 36° C it is open. The addition of TuTs at 0.1 M KCl stimulates rRNA synthesis from the closed

promoter at 28 and 34° C, a five-fold increase being observed at 34° C (Table 1). As TuTs does not affect total RNA synthesis significantly the proportion of rRNA in the transcript is concomitantly increased. Therefore TuTs here alters the specificity of transcription. In contrast, TuTs has no effect on synthesis from the open rRNA promoter at 38° C.

At 0.01 M KCl the rate of rRNA synthesis by holoenzyme alone is substantially higher than that at 0.1 M KCl regardless of temperature⁹. Nevertheless, the response of rRNA synthesis to TuTs at the lower salt concentration resembles that at the higher. At 38° C TuTs inhibits rRNA synthesis by ~40%. Yet, at both 28 and 34° C, TuTs stimulates rRNA synthesis two to three-fold. Indeed, at 28° C with TuTs, rRNA comprises ~25% of the transcript—a figure that should be compared with the maximum *in vivo* proportion of 40–50%.

A variation in the rate of synthesis of a specific RNA species should be correlated with a similar variation in the types of 5' terminal RNA sequences. At the simplest level we would expect to observe a change in the ratio of number of rRNA chains initiated with pppA to those initiated with pppG, as most RNA molecules synthesized *in vitro* are initiated with a purine nucleotide^{10,11}. Measurement of the incorporation of ATP and GTP into the 5' terminal position of *E. coli* RNA synthesized at 34° C and 0.1 M KCl revealed that TuTs selectively stimulates pppG initiations and slightly inhibits pppA initiations (Table 2). rRNA synthesis therefore correlates with pppG initiations. A similar correlation is observed on comparing the termini of *E. coli* RNA species synthesized at 0.01 M and 0.1 M KCl. pppA initiations are relatively independent of salt concentration but pppG initiations and rRNA

Table 1 Effect of TuTs and ppGpp on rRNA Synthesis

KCl concentration		Total synthesis	28° C rRNA synthesis	rRNA %	Total synthesis	34° C rRNA synthesis	rRNA %	Total synthesis	38° C rRNA synthesis	rRNA %
0.01 M	E σ	24,300	3,370	13.7	37,600	3,520	9.6	46,800	4,760	10.2
	E σ +0.5 mM ppGpp				21,300	1,890	8.9	31,400	3,050	9.7
	E σ +TuTs	35,300	9,020	25.6	39,200	6,330	16.3	45,300	3,080	6.8
	E σ +TuTs+0.5 mM ppGpp				19,700	2,030	10.3	29,200	2,660	9.1
0.1 M	E σ	8,200	590	7.2	17,400	420	2.4	21,200	2,460	11.6
	E σ +0.5 mM ppGpp				13,800	320	2.3	10,900	1,320	12.1
	E σ +TuTs	12,100	2,280	18.8	16,800	2,010	11.9	20,600	2,320	11.4
	E σ +TuTs+0.5 mM ppGpp				10,200	330	3.2	14,300	1,500	10.5
	E σ				19,700	360	1.8			
	E σ +Tu				15,200	1,410	9.3			
	E σ +Ts				25,400	660	2.6			

The reaction mixtures for *in vitro* RNA synthesis contained in a volume of 0.2 ml, 0.04 M Tris-HCl, pH 7.9, at 20° C, KCl as indicated, 0.01 M MgCl₂, 0.0002 M dithiothreitol, 0.001 M EDTA, 0.25 mM ATP, 0.25 mM CTP, 0.25 mM GTP, 0.0045 mM UTP specific activity 13,000 Ci mol⁻¹, 70 μ g ml⁻¹ *E. coli* DNA and ppGpp as indicated. The reaction mixtures were incubated for 10 min and RNA synthesis was started by the addition of RNA polymerase (at 40 μ g ml⁻¹) together with TuTs (at 8 μ g ml⁻¹), Tu (10 μ g ml⁻¹) or Ts (5 μ g ml⁻¹) as appropriate. The incubation was continued for 15 min and RNA synthesis was stopped by the addition of DNase and the RNA processed as previously described⁹. rRNA was determined by the competition by unlabelled rRNA for labelled *in vitro* RNA hybridized to denatured *Vibrio* DNA⁹. The variation about the mean in the determined values of rRNA synthesis is normally within ± 150 c.p.m. at low levels of synthesis (<700 c.p.m.). TuTs was either isolated from Q β replicase (the gift of Dr T. Blumenthal) by the method of Kamen¹⁹ or was prepared directly from uninfected cells. The ψ r activity of TuTs prepared by these different methods was identical. Separated Tu and Ts were the gift of Drs Harshmann, Miller and Weissbach. The Tu contamination of Ts was <1%, the Ts contamination of Tu was <0.1%. RNA polymerase holoenzyme was >95% pure and contained no detectable polypeptides of the same electrophoretic mobility as either Tu or Ts.

synthesis are markedly enhanced at low KCl concentrations (A. A. T., unpublished observations).

Unique Response?

Is the response of rRNA synthesis to TuTs unique? With T4 DNA as a template, TuTs inhibits transcription below 0.1 M KCl and decreases RNA synthesis by 60% at 0.01 M KCl (Fig. 1) but above 0.1 M KCl TuTs has no significant effect. This response thus closely resembles that of rRNA synthesis at 38° C but differs from that of rRNA synthesis at 34° C. This suggests that the conformation of the rRNA promoters, like that of the T4 promoters⁹, is insensitive to changes in KCl concentration in normal conditions of *in vitro* RNA synthesis. I conclude that TuTs acts as a transcription specificity factor stimulating RNA synthesis from the closed rRNA promoter at all tested KCl concentrations and inhibits synthesis from both T4 promoters and the open rRNA promoter at 0.01 M KCl.

Table 2 5' Terminus of RNA Synthesized with and without TuTs

	$\gamma^{32}\text{P-GTP}$	$^3\text{H-UTP}$
E σ	540	8,130
E σ +TuTs	1,420	12,380
	$\gamma^{32}\text{P-ATP}$	
E σ	730	8,490
E σ +TuTs	650	13,110

The reaction mixtures were as described in Fig. 1 except that either ATP or GTP prepared by the method of Glynn and Chappell²⁹ was ^{32}P -labelled in the γ phosphate at a specific activity of 1,700 Ci mol⁻¹. UTP was ^3H -labelled at a specific activity of 160 Ci mol⁻¹. KCl was 0.1 M. RNA synthesis was for 10 min at 34° C following a preincubation of DNA for 10 min at 34° C. Incorporation of ^3H and ^{32}P into RNA was determined as previously described³⁰.

When Tu and Ts are tested individually for their ability to increase transcription from the closed rRNA promoter, only Tu preferentially enhances rRNA synthesis (Table 1). Tu is therefore sufficient for the control of specificity.

RNA Polymerase Conformations

How does TuTs influence the specificity of transcription? The factor could bind to the DNA template and consequently either activate or deactivate it. Alternatively, TuTs could bind to RNA polymerase and alter its initiation specificity.

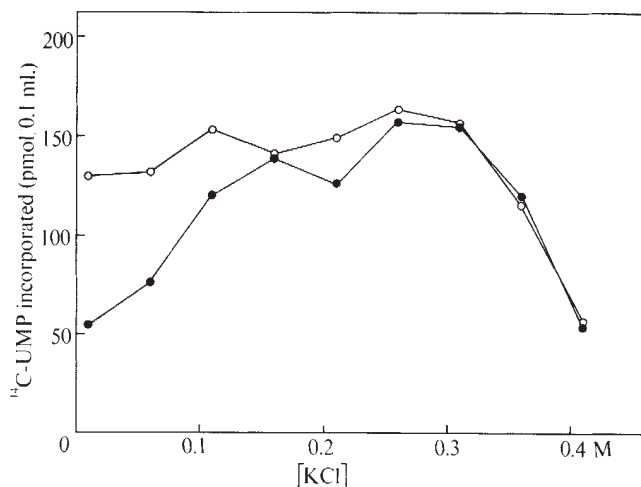


Fig. 1 Effect of TuTs on T4 RNA synthesis. The reaction mixtures were as in Table 1 except that ^{14}C -UTP was 0.025 mM at a specific activity of ~ 47 Ci mol⁻¹ and T4 DNA at 20 $\mu\text{g ml}^{-1}$ replaced *E. coli* DNA. All reaction components were mixed at 0° C and incubated for 10 min at 37° C. Incorporation of ^{14}C -UMP into RNA was determined by trichloroacetic acid precipitation. ○, E σ ; ●, E σ +TuTs.

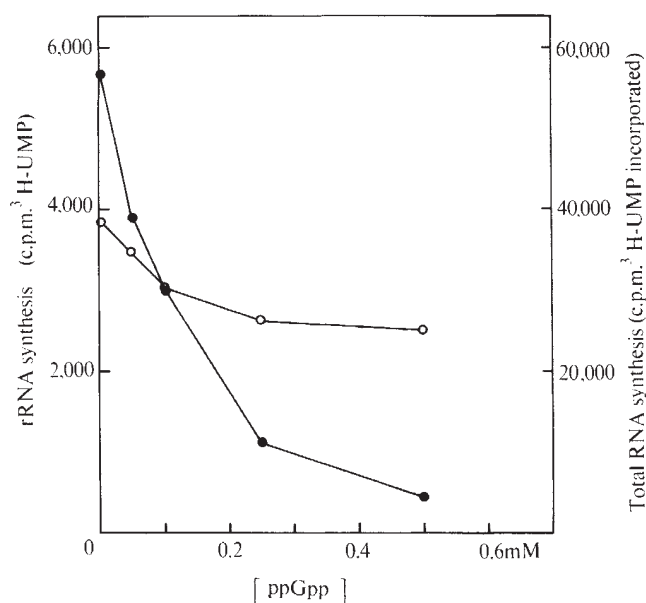


Fig. 2 Inhibition of TuTs-dependent rRNA synthesis by ppGpp. The reaction mixtures were as in Table 1 except that ^3H -UTP concentration and specific activity were 0.0015 mM and 41,000 Ci mol⁻¹ respectively. The preincubation of DNA was for 10 min at 34° C and RNA synthesis was for a further 10 min at 34° C. rRNA (●) and total RNA (○) syntheses were measured as described elsewhere⁹.

Attempts to demonstrate binding of TuTs directly to either *E. coli* DNA or polymerase holoenzyme have so far yielded negative results (A. A. T., unpublished observations), although 5% of the Tu activity in a crude extract of *E. coli* cosediments with a fraction of the RNA polymerase activity¹². The transcriptional properties of this crude polymerase closely resemble those of a reconstituted mixture of holoenzyme and TuTs. This evidence indicates a direct effect of TuTs on RNA polymerase.

The initiation specificity of RNA polymerase holoenzyme, either alone or in combination with TuTs, is strongly dependent on the KCl concentration^{9,13}. Particularly striking is the tenfold higher rate of synthesis by holoenzyme from the closed rRNA promoter at 0.01 M KCl compared with 0.1 M KCl. The selective enhancement of this synthesis by TuTs is most apparent at the higher KCl concentration. In contrast, synthesis by holoenzyme from the open rRNA promoter, or from T4 DNA, is relatively insensitive to variations in KCl concentration. In these cases, TuTs inhibits synthesis substantially at 0.01 M KCl but has little effect at 0.1 M KCl.

The KCl-dependent variation in the initiation specificity of holoenzyme correlates with a change in the physical properties of the enzyme. Below 0.12 M KCl holoenzyme is an equilibrium of protomeric and dimeric forms, the dimeric form becoming predominant as the KCl concentration is decreased¹⁴. A simple model that accounts for these observations proposes that RNA polymerase can exist in two conformational states, (E σ)_m and (E σ)_s (formerly termed (E σ)_A and (E σ)_G respectively)^{15,16}. In this model (E σ)_m initiates RNA synthesis efficiently at mRNA promoters, as exemplified by T4 promoters and at open rRNA promoters, while (E σ)_s initiates efficiently only at closed rRNA and tRNA promoters. The polymerase forms are assumed to be in equilibrium. At high KCl concentrations, (E σ)_m is strongly favoured while at low KCl concentrations neither form is favoured. The role of TuTs is to stabilize the (E σ)_s form. Consequently, whereas in the presence of TuTs, neither form is favoured at high KCl concentrations, at low concentrations (E σ)_s is the dominant form. Thus, in the extreme situations when one form of the polymerase is limited, synthesis of the corresponding RNA is markedly restricted. Although this model is sufficient to

explain the data, I cannot exclude more complicated models proposing that holoenzyme may exist in more than two initiation conformations.

Two independent lines of evidence indicate that RNA polymerase can exist in different conformational states with differing initiation specificities. First, a mutant RNA polymerase, ts103 (ref. 17), compared with parent enzyme, synthesizes rRNA more efficiently from the closed rRNA promoter and much less efficiently from the open rRNA promoter (Travers, to be published). ts103 polymerase thus behaves as though the equilibrium between $(E\sigma)_m$ and $(E\sigma)_s$ is shifted in favour of $(E\sigma)_s$ as a consequence of the mutation. Second, the physical and functional heterogeneity of RNA polymerase in *E. coli* crude extracts^{12,18} indicates that different forms of holoenzyme can be stabilized by interaction with other proteins, including Tu.

Clear analogies exist between the role of TuTs in RNA phage replication and its proposed involvement in transcription. In both cases TuTs interacts directly with other polypeptides to form either the phage replicase^{8,19,20} or a complex with polymerase holoenzyme. In neither case does TuTs seem to bind independently to the template. Also, both in transcription and in phage replication, TuTs stimulates the synthesis of RNA molecules initiated with pppG^{21,22}. As TuTs is required for the use of poly (C) templates by replicase⁸ it seems probable that TuTs again acts by stabilizing an initiation conformation of the enzyme rather than by recognizing a tertiary structure resembling tRNA.

Inhibition of rRNA Synthesis by ppGpp

Both Tu⁸ and RNA polymerase²³ are inhibited by ppGpp. Yet selective inhibition of rRNA synthesis by 0.5 mM ppGpp is only observed below 35° C in the presence of TuTs (Table 1). Thus at 34° C TuTs dependent rRNA synthesis is half inhibited by 0.2 mM ppGpp (Fig. 1), a figure very close to the K_i for the inhibition by ppGpp of Tu-dependent binding of aminoacyl tRNA to ribosomes (A. A. T., unpublished observations). The proportion of rRNA in the transcript falls from ~15% in the absence of ppGpp to ~2% at 0.5 mM ppGpp. In contrast, rRNA synthesis by holoenzyme alone, from either open or closed rRNA promoters, is inhibited by ppGpp only to the same extent as total RNA synthesis (Table 1). Similarly, ppGpp does not preferentially inhibit RNA synthesized from the open rRNA promoter in the presence of TuTs. I conclude that the closed rRNA promoter and TuTs are prerequisites for selective control of rRNA synthesis by ppGpp.

Table 3 Effect of TuTs and ppGpp on tRNA Synthesis

	Input RNA (c.p.m.)	Hybridized RNA (c.p.m.)		Δ
		-tRNA	+tRNA	
E σ	58,100	11,462	11,833	-371
E σ +0.4 mM ppGpp	47,600	9,338	9,180	158
E σ +TuTs	60,300	11,220	9,830	1,390
E σ +TuTs+0.4 mM ppGpp	40,700	7,327	7,632	-305

The reaction mixtures for RNA synthesis were as described in Table 1 except that the labelled precursor was $\alpha^{32}\text{P}$ -GTP at a concentration and specific activity of 0.004 mM and 8,500 Ci mol⁻¹. UTP was 0.25 mM and $\phi 80\text{psu}^{+}_{\text{III}}$ (doublet) DNA at 10 $\mu\text{g ml}^{-1}$ replaced *E. coli* DNA. KCl was 0.1 M. All reaction components were mixed at 0° C and incubated for 10 min at 37° C. The RNA was then processed as previously described. Synthesis of tRNA was determined by hybridizing the labelled *in vitro* transcript in 0.2 ml 2 $\times$ SSC to denatured $\phi 80\text{psu}^{+}_{\text{III}}$ (doublet) DNA at 5 $\mu\text{g ml}^{-1}$ in the presence and absence of unlabelled tRNA^{Tyr} at 0.15 $\mu\text{g ml}^{-1}$ for 4 h at 67° C.

The synthesis of another stable RNA species is also inhibited by ppGpp. In the absence of TuTs no *in vitro* synthesis of tRNA^{Tyr} from $\phi 80\text{psu}^{+}_{\text{III}}$ DNA is detectable (Table 3). Yet with TuTs, 10% of the labelled transcript hybridized to

denatured $\phi 80\text{psu}^{+}_{\text{III}}$ DNA is competed by unlabelled tRNA^{Tyr} although with TuTs and 0.4 mM ppGpp to tRNA synthesis is detected. TuTs is again stimulating the synthesis of an RNA molecule that is initiated with pppG, both *in vivo*²⁴ and *in vitro*²⁵.

Control of Stable RNA Synthesis

In wild-type bacteria, the net synthesis of stable RNA species is preferentially inhibited relative to mRNA synthesis whenever the bacteria are starved for an amino acid or their growth rate is otherwise restricted. This independent inhibition of stable RNA accumulation is inversely correlated with the concentration of ppGpp, which behaves like a non-competitive inhibitor with a K_i of 0.1–0.2 mM⁵. *In vitro*, the only mode of rRNA synthesis described here that is selectively inhibited by ppGpp is TuTs-dependent synthesis from the closed form of the rRNA promoter. In this case the K_i for the inhibition of rRNA synthesis by ppGpp is ~0.2 mM, and approximates closely to the characteristics of the *in vivo* situation.

Two other lines of evidence suggest that the rRNA promoter is normally closed *in vivo* and that rRNA synthesis can be regulated by controlling the activity of $(E\sigma)_s$ through the action of ppGpp on TuTs. First, the ts103 mutant fails to restrict rRNA synthesis *in vivo* to the same extent as its parent bacterial strain (Jacobson and Gillespie, personal communication). *In vitro* this situation is mimicked by the comparative rate of rRNA synthesis by mutant and parent holoenzymes from the closed rRNA promoter only. Second, the putative TuTs-holoenzyme complex in crude extracts of *E. coli* is detectable neither when extracts are prepared from stationary phase cells, which synthesize rRNA very slowly, nor when the complex is isolated in the presence of ppG, an analogue of ppGpp¹². Another mechanism proposed for the control of rRNA synthesis invokes a repressor binding to the DNA^{7,26}. There is no positive evidence for the existence of such an entity.

The characteristics of the *in vitro* regulation of stable RNA synthesis described here duplicate the major features of the control of stable RNA accumulation *in vivo*, suggesting that the rate of accumulation reflects principally a control on synthesis rather than a control on degradation²⁷. This control of stable RNA synthesis by TuTs, a protein synthesis elongation factor, and by ppGpp, a metabolic product of the ribosome²⁸, emphasizes the coupling between protein synthesis and the transcription of those RNA species destined to become a stable part of the protein synthesizing machinery of the cell.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Long Baseline Interferometry of the Seyfert Galaxy 3C84

A CONTINUING programme to measure the visibility of radio sources and monitor the possible variability in their angular structure is in progress by collaboration between the Algonquin Radio Observatory, Lake Traverse, Ontario (operated by the National Research Council of Canada) and the Field Station at Chilbolton, England (operated by the Radio and Space Research Station of the UK Science Research Council). As an initial result the visibility curve for 3C84, taken in November 1972, is presented here, with two possible interpretations.

A long baseline interferometer was formed by the 45-m telescope at the Algonquin Radio Observatory and the 25-m telescope at Chilbolton. At the operating frequency of 10,680 MHz, the baseline of 5,265 km represents a separation between telescopes of 187×10^6 wavelengths. Each station received left-hand circular polarization. Cooled parametric amplifiers, local oscillators locked to hydrogen masers and video tape recorders¹ formed the essential components of the receiving systems.

A plot of the correlated flux density against hour angle is shown in Fig. 1, each point representing a 5-min average. The flux density scale was derived from two independent methods which agreed within 15%. In the first method the r.m.s. noise in flux units was calculated from the system parameters; a measurement of the signal-to-noise ratio of the fringes then gave the fringe amplitude in flux units. In the second method, c.w. signals of known flux levels were inserted at each end and processed in the same manner as signals from the radio source. The flux scale derived by these methods gave a peak correlated flux density of 2.5 f.u. compared with a zero baseline flux of 50.0 f.u. (W. J. Medd, B. H. Andrew and G. A. Harvey, private communication). There are several factors which might diminish the correlation of the signals from the telescopes, not all of which affect the c.w. calibration method,

therefore the peak flux may be higher. An upper limit of 7.5 f.u. for the correlated flux from 3C84 can be obtained from our observations of BL Lac by assuming that its peak correlated power represents the zero baseline flux. We note, however, that BL Lac is heavily resolved at some hour angles, and may be partially resolved at all hour angles with this baseline.

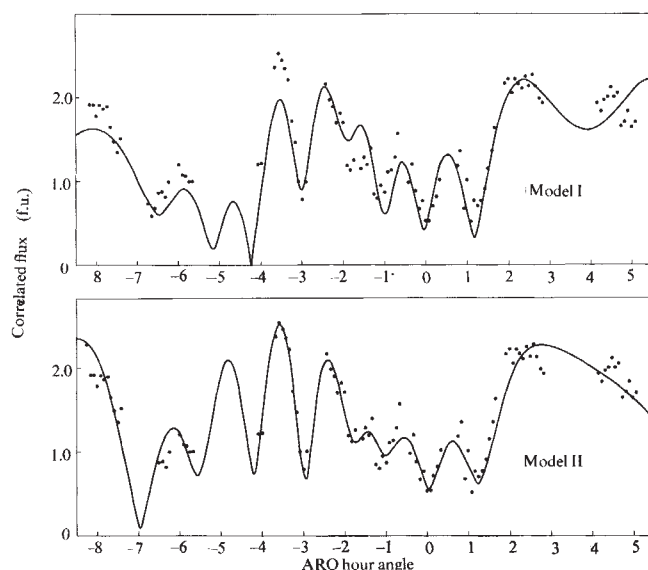


Fig. 1 Visibility curve for 3C84 (November 1972). The points represent averaged values of correlated flux plotted against hour angle and are shown with the best-fit visibility curves derived from model I and model II.

The parameters of two models, fitted to the data by an iterative method, are presented in Table 1. Model I consists of a completely resolved component containing most of the flux, and four unresolved components (diameters < 0.0002 arc s). Model II consists of four heavily resolved components, for

Table 1 Model Parameters

Parameter	Component	Model I				Model II			
		1	2	3	4	1	2	3	4
Flux (f.u.)		0.98	0.42	0.72	0.31	18.7	17.2	8.4	5.6
Major axis*		—	—	—	—	1.36	1.64	1.30	1.40
Minor axis*		—	—	—	—	1.19	0.90	0.98	1.09
Position angle of major axis (°)		—	—	—	—	364	178	369	350
Length of position vector†		3.49	3.10	2.09	1.39	3.27	3.12	1.90	0.36
Position angle of position vector (°)		174	354	174	341	357	171	352	334

* Width at half-intensity in arc ms.

† Angular distance from the arbitrary origin in arc ms.

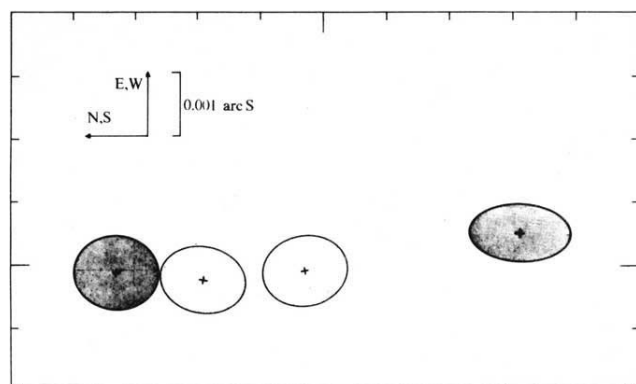


Fig. 2 Model II. The ellipses represent the size of the components at half intensity and the shading indicates their relative strength.

which Gaussian brightness distributions and elliptical cross-sections were assumed. The visibility curves for these models are presented in Fig. 1. A drawing of model II is shown in Fig. 2: the ellipses represent the sizes of the components at half intensity, and the outer two components are about twice as bright as the inner pair, as indicated by the shading. In both models the components seem to be roughly aligned in a plane normal to the sky, which may indicate some preferred orientation within the source. Attempts to fit the data with models containing less than four small components resulted in unacceptably large deviations between the observed and calculated visibilities.

It is not possible to decide between unresolved and heavily resolved components with observations published to date. If model II is assumed then the peak brightness temperature is 3×10^{11} K. This value is close to the upper limit of about 10^{12} K imposed by the onset of inverse-Compton absorption². If the turnover frequency in the spectrum of 3C84 is taken to be 10 GHz for all four components, then the magnetic fields lie between 10^{-1} and 10^{-2} gauss. This supports the conclusion of Kellermann and Pauliny-Toth² that 3C84 is among those compact sources having exceptionally high magnetic fields.

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Angular Diameters of Quasars of Unusual Colour

Two neutral-coloured stellar objects, OH471 and OQ172, have been shown to be QSOs with redshifts of 3.40 and 3.53 respectively^{1,2}. This has stimulated interest in other radio sources identified with stellar objects which lack the ultraviolet or blue colour excesses normally associated with quasars. Browne and McEwan³ have published a list of objects which, though blue, show no ultraviolet excess; I. W. A. B., J. H. Crowther and R. L. Adgie have prepared a list (unpublished) of sources identified with neutral coloured objects, which includes both OH471 and OQ172. Sources of both types have been put forward as possible high redshift QSOs. We have measured the radio angular diameter of the sources on both lists, and of four further objects which may possibly be similar. These objects^{4,5}, OJ287, W Com, ON235 and BL Lac, show no spectral lines but resemble OH471 and OQ172 in colour.

The observations were made at Jodrell Bank with the Mark II-Mark III interferometer which has a baseline of 23.8 km, giving a maximum projected spacing at 1,660 MHz of 132,000 wavelengths. Each source was observed with this instrument for at least 10 min at two widely separated hour angles, one before and one after meridian transit. In each case the projected spacing for the early hour angle was close to the maximum value. The r.m.s. noise error after 10 min integration is 0.03 f.u.

The flux densities at 1,660 MHz have not been measured directly, and have therefore been predicted from radio spectra compiled from published flux density measurements. The spectra were also used to classify the sources into those with steep spectra and those with a centimetric excess. Following Andrew et al.⁶ we take type CE as a spectrum with a maximum in the centimetre region or with spectral index $\alpha > -0.3$ (where $S \propto \nu^\alpha$). Type S spectrum has $\alpha \lesssim -0.5$.

The visibility, γ , of each source at each of the two hour angles (Table 1) is obtained by dividing the measured amplitude by the predicted flux density. The chief error in γ arises from the uncertainty in the predicted flux density.

Table 1 contains a large proportion of CE objects. The reasons for this, at least for the objects of neutral colour, will be discussed elsewhere. Such sources⁶ are frequently variable, which leads to an added uncertainty in the predicted flux density, in a few cases amounting to about a factor of 2. Sources in Table 1 with visibilities appreciably greater than 1.0 must be in this category.

We do not propose to fit model brightness distributions, because two spot measurements of γ are insufficient for this process to be meaningful. We divide the sources into those for which $\gamma \gtrsim 0.5$ at both hour angles and those for which $\gamma < 0.5$. The value $\gamma = 0.5$ corresponds, for a double source, to an angular size of 0.5 arc s. Sources are labelled "Compact" or "Extended" according to this classification in Table 1. None of the 40 sources observed was completely resolved ($\gamma = 0$), showing that even the extended sources contain some structure at about the 1 arc s scale.

Most sources (thirty-six out of forty) are compact, probably with sizes less than 0.5 arc s. As a test of the suggestion that these objects are QSOs of unusual colour, we may compare them with a sample of QSOs having the characteristic ultraviolet excess.

Miley⁷ has published angular size measurements of such a sample to a limit of 7 arc s. We show in Table 2 a comparison of the two lists, separated according to the radio spectrum into the categories CE and S. Although Miley's dividing line is set at 7 arc s, whereas ours is set at 0.5 arc s, the fraction of compact sources in our list seems somewhat higher for each spectral category. This difference may partly be due to selection effects.

The principal impression gained from Table 2 is that the

Table 1 Observations

Source	Name	Reference	Predicted flux density (f.u.)	Radio spectral type	Measured visibility before transit	Measured visibility after transit	Structure
0024+34	OB338	4	0.90	CE	1.03	1.12	Compact
0035+36	GC	4	0.73	S	0.29	0.27	Extended
0105-008	PKS	3	0.85	S	0.94	—	Compact
0205-010	PKS	3	0.45	CE	0.73	—	Compact
0234+09	OD058	4	0.78	S	1.19	1.04	Compact
0256+07	OD094.7	4	1.05	CE	0.61	0.80	Compact
0458-02	PKS	3	1.80	CE	1.14	1.06	Compact
0529+07	OG050	4	2.00	CE	0.76	0.78	Compact
0642+44	OH471	4	1.90	CE	0.92	0.92	Compact
0723-008	OI-039	4	3.00	CE	0.63	0.77	Compact
0735+17	PKS	4	2.20	CE	0.74	0.91	Compact
0743-006	OI-072	4	0.95	CE	1.12	1.07	Compact
0805+04	4C05.34	3	0.57	S	0.81	0.56	Compact
0808+019	PKS	3	0.65	CE	0.80	0.74	Compact
0819-032	PKS	3	0.45	CE	0.76	0.65	Compact
0851+20	OJ287	6	2.00	CE	1.26	—	Compact
1119+18	OM133	4	0.65	CE	0.86	0.46	Compact
1134+01	PKS	4	0.90	S	0.47	0.39	Extended
1215+30	ON235	5	0.55	CE	0.58	0.55	Compact
1219+2	W.Com	5	0.60	CE	1.72	1.75	Compact
1427-01	PKS	4	0.60	S	0.65	0.87	Compact
1442+10	OQ172	4	2.30	CE	1.07	1.04	Compact
1509+022	PKS	4	0.85	S	0.29	0.25	Extended
1514+19	GC	4	0.50	CE	1.08	0.86	Compact
1604+15	OS108.2	4	0.60	CE	0.22	0.44	Extended
1656+05	OS094	4	1.50	CE	1.10	0.95	Compact
1705+018	PKS	3	0.50	CE	1.04	1.14	Compact
1749+09	OT081	4	1.60	CE	0.65	0.65	Compact
1954+51	OV591	4	1.50	CE	1.08	1.10	Compact
2003-025	PKS	3	1.90	S	1.10	1.12	Compact
2022+54	OW538	4	1.20	CE	1.01	0.85	Compact
2121+05	OX036	4	0.65	CE	2.04	2.14	Compact
2136+14	OX161	4	1.00	CE	1.26	1.28	Compact
2141+17	OX169	4	0.35	CE	0.96	1.20	Compact
2201+42	BL.Lac	6	4.50	CE	0.79	0.78	Compact
2207+020	PKS	3	0.55	S	0.75	0.58	Compact
2254+07	OY091	4	0.50	CE	1.22	1.28	Compact
2256+017	PKS	3	0.45	CE	0.82	—	Compact
2320-035	PKS	3	0.40	CE	1.95	—	Compact
2351-006	PKS	3	0.45	CE	1.11	—	Compact

Table 2 Relative Numbers of Compact and Extended Sources

	CE radio spectrum		S radio spectrum	
	Compact	Extended	Compact	Extended
Miley's list	38	9	15	60
Present list	30	1	6	3

angular size depends markedly on radio spectral index: a very high proportion of CE sources in both lists is compact. In contrast, the optical colour, whether characterized by *U*-excess, *B*-excess or neutral colour, seems to have relatively little connexion with the size of the source.

OH471 ($z=3.40$) and OQ172 ($z=3.53$) both have angular sizes of less than $\frac{1}{2}$ arc s, which in a Friedmann universe with $H_0=50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0=+1$ implies a linear diameter less than 2.5 kpc.

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Alpine Glacial Features of Mars

EVIDENCE of past alpine glacial erosion in the south polar region of Mars appears in two photographs taken by Mariner 9 in early 1972. Although no glacial remnants show up in either photograph, the aretes, U-shaped valleys, and other glacial evidences closely resemble the ice-free features of the Cascade Mountains in the northwestern United States. Continental glaciers that exist near the Martian poles have been described previously^{1,2}. Axial precession and changes in the orbital eccentricity are thought to be the cause of climatic variations on the Martian surface. Thus, the alpine glaciers responsible for the erosion of the features described herein could have recently disappeared because of a warming trend that did not eliminate the polar ice caps.

Mariner 9 photograph 182-27 shows a mountainous region at -76.6° latitude and 72.7° longitude that is about 120 km

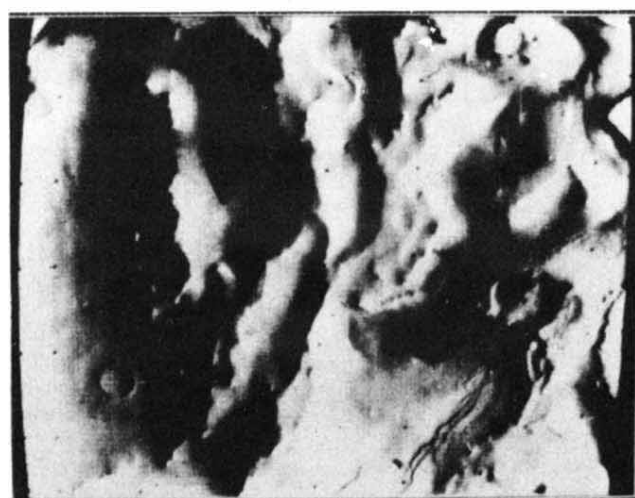
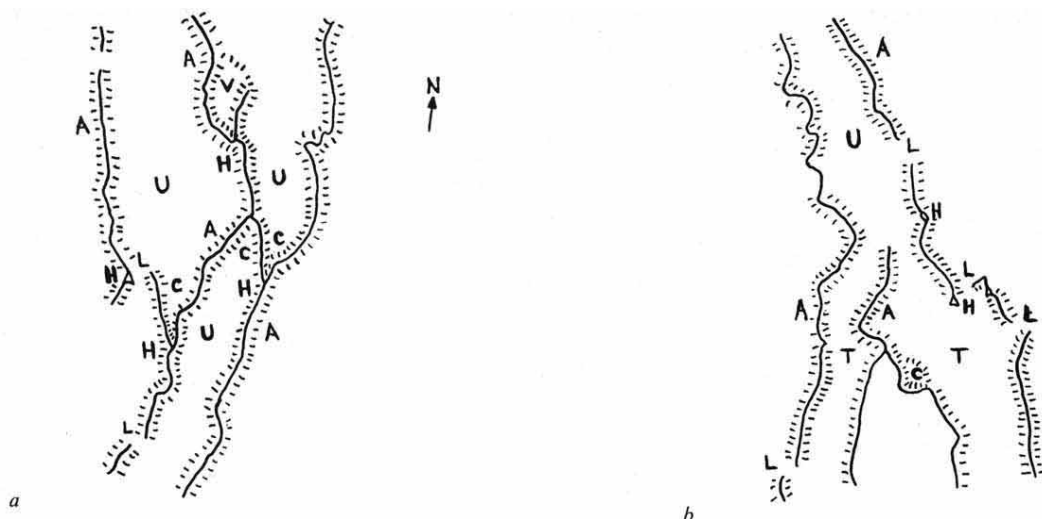


Fig. 1 *a*, Alpine glacial features on Mars. Two north-sloping U-shaped valleys are separated from a south-sloping U-shaped valley by a Z-shaped cirque-arete. Horns, cols and a hanging valley are present. (Mariner 9 photograph P182-27.) A, Arete; C, cirque; L, col; V, hanging valley; T, tributary valley; U, U-shaped valley. *b*, Alpine glacial features on Mars. Two tributary U-shaped valleys merge to form a northwesterly sloping main U-shaped valley. Aretes, cols, horns and a cirque hanging valley are present. (Mariner 9 photograph P227-19.) (There is no sign of actual glaciers in either photograph.)

along its north-south dimension. Alpine glaciation seems to be the dominant geological factor in shaping the terrain of this area. Three U-shaped valleys are present in the centre with sharp aretes, cirques, and horns separating them. One valley slopes southward and begins as a cirque separating the other two north-sloping cirques with a Z-shaped cirque-arete.

A classic triangular, concave-sloped horn appears at the junction of the three chief cirques at the northernmost tip of the south-sloping valley. A col and several small horns are present near the head of the western north-sloping valley. Also, a hanging valley is present on the west wall of the eastern north-sloping valley.

Photograph 227-19 (-81.9° latitude, 56.0° longitude, and about 80 km along its north-south dimension) shows additional evidence of past alpine glaciation. Two tributary U-shaped valleys merge to form a single large U-shaped valley that slopes in a north-northwesterly direction. A prominent arete broken by several cols forms the eastern wall of both the main valley and the eastern tributary. Several horns are also present on this arete. Although the main cirques of each tributary do not appear in the photograph, a small cirque and hanging valley show up on the southwest wall of the eastern tributary valley. The westernmost arete has undergone more extensive erosion than the other valley walls.

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Sizes of Conical Volcanoes

THE shield volcano Mauna Loa in Hawaii is the world's highest mountain, 9,144 m high¹, if the portion below sea level is considered in addition to the exposed 4,170 m. Why, then, are the world's highest mountains not land volcanoes? We have investigated the heights and volumes of land volcanoes to try to establish what factors prevent their development to heights comparable with Mauna Loa. Data on these elementary parameters are scarce. Generally, only the height of a volcano's summit above sea level is quoted, not the height above geological base level. Volumes of individual volcanoes are rarely given.

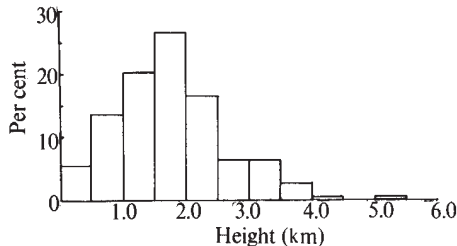


Fig. 1 Height/frequency distribution for 181 conical composite volcanoes. Data from *Catalogue of Active Volcanoes of the World*².

For simplicity, collection of data was confined to conical volcanoes listed in the *Catalogue of Active Volcanoes of the World*², for which the height of the cone itself is quoted. The height/frequency distribution of 181 cones (Fig. 1) is a unimodal one with a peak in the range 1,500 to 2,000 m, indicating that the "average" conical volcano is relatively low. The highest volcano cone is probably the Nevado Ojos del Salado in Chile, which rises 7,010 m above sea level, but only about 3,600 m above its base. The biggest cone is probably Kilimanjaro, although there is some dispute as to the precise height of its base. Consideration of the way in which a conical volcano increases in volume is informative. A simple scoria cone has the profile of a truncated cone with sides making an angle of 33° with the horizontal; this is the angle of rest for loose scoria. Such true cones rarely exceed 500 m in height. As the volcano grows its shape progressively departs from the conical, and adopts a profile which can be described by an equation of the form:

$$h = m \log r + c$$

where h =height, r =radius and m and c are constants.

We used this expression to calculate the volume of the cone of Mayon volcano in the Philippines, 2,435 m high and widely regarded as the most symmetrical volcanic cone³. With only small error, the same value (112 km³) can be obtained by assuming a truly conical shape, but with sides making an angle of only 18° with the horizontal. We call this angle the nominal cone angle, and assume that, during cone development, the value of this angle progressively decreases with height, from 33° at 750 m to 18° at 2,435 m. This assumption enables us to calculate an approximate value for the volume of a cone when only the height is quoted (Fig. 2).

The significance of this is that, given a constant rate of eruption, the time of formation of volcanic cones will follow a similar curve. Few data are available on the rates of eruption of conical volcanoes. Mauna Loa has added approximately 0.03 km³ of basalt lavas to its bulk annually⁴. If the rate of eruption of an "average" andesitic conical volcano were only 0.1% of this, a 3,500 m cone would form in only 13 m.y. In some cases, rates of

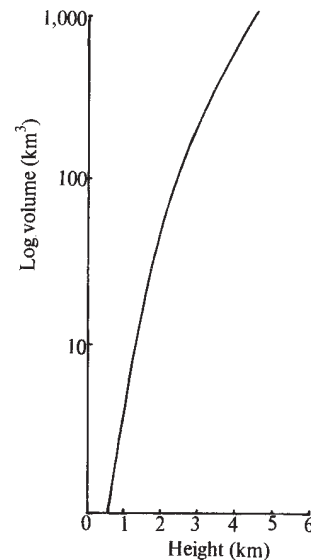


Fig. 2 Curve to illustrate rate of increase of volume of conical volcanoes with height of cone.

construction are such that this height would be exceeded in a much shorter period. Gass⁵ has suggested that Tristan da Cunha, a 5,700 m high cone built above the ocean floor, grew in only 3 m.y.

Because there are so few land volcanoes higher than 3,500 m, it seems that some process must intervene to prevent attainment of greater heights. We consider that this could be caldera formation.

Many major calderas are known to occupy the sites of pre-existing high conical volcanoes. The classic example is Crater Lake caldera, believed to have formed after an eruption of Mt Mazama, originally some 3,500 m high⁶. We have plotted the diameter/frequency distribution curve for the 116 calderas tabulated by Simpson⁷ (Fig. 3). Significantly, the peak lies in the range 2 to 5 km, implying that the parent volcanoes of these "average" calderas were large, perhaps 3,000 to 4,000 m high. Clearly, not all calderas originate from conical volcanoes; this seems to be true for only two of the six types recognized by McBirney and Williams⁸. Those calderas with diameters greater than 7 or 8 km are almost all associated with eruptions of large volumes of either pyroclastic flows (Valles type) or basaltic lavas (Masaya and Hawaiian types), and are not necessarily related to pre-existing conical volcanoes.

We will not speculate on the mechanistic links between

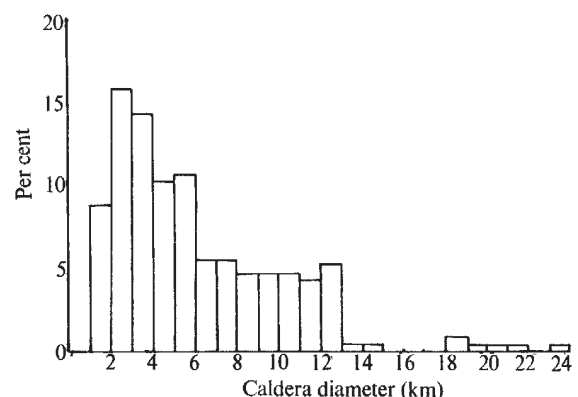


Fig. 3 Diameter/frequency distribution for 116 calderas. Data from Simpson⁷.

conical volcanoes and caldera formation, although there may possibly be a limiting value to the height of the magma column that can be sustained by a conical volcano. We suggest, however, that caldera formation is the most likely reason for land volcanoes rarely exceeding heights greater than 3,500 m. If correct, this has some interesting implications. Calderas are more abundant in some parts of the world than others. They are abundant in the Japanese part of the circum-Pacific volcanic belt, but almost entirely absent in the Andean part. In the active volcanic belt of the Central Andes there appear to be no volcanic rocks older than about 10 m.y. (refs. 9, 10), yet there are many conical volcanoes about 3,000 m high. We suggest that, if activity in the area continues, caldera formation may affect many of them. Generalizing, we suggest that where any conical volcano has reached a height of over 3,000 m, caldera formation will be the next stage in its evolution. In this context it is interesting that the major Plinian eruptions which have accompanied the very few historic cases of caldera formation have all affected volcanoes with no previous record of historical activity (for example, Vesuvius (Somma) AD 79, Katmai 1912). The next major caldera-forming eruption may well involve therefore a volcano now generally considered "extinct".

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Association of Hydrocarbons and Mineral Particles in Saline Solution

WE report evidence confirming the suggestion that the partial dissolution in seawater of fuel oil hydrocarbons is important in the degradation of this petroleum product in the marine environment¹. Our experiments show that hydrocarbon solubility affects uptake and retention of fuel oil by marine sediments and that organic matter in sediments reduces the incorporation of fuel oil into the sediment samples investigated.

Our experiments were done in litre volumes of saline solution (30 g NaCl kg⁻¹ solution) adjusted to pH 8.0. The experimental method has been described in ref 2. The recovery of sorbed hydrocarbons from bentonite clay (<44 μ m) was 100 $\pm$ 3.1% for eicosane (n -C₂₀H₄₂), 101 $\pm$ 3.1% for anthracene (three fused benzene rings, C₁₄H₁₀), and 92 $\pm$ 3.1% for hexadecane (n -C₁₆H₃₄). A maximum coefficient of variation³ of 3.1% was found for the entire procedure. The

reported analyses have been corrected for the natural hydrocarbons found in the clays and sediments.

One hundred micrograms of each of the hydrocarbons dissolved in 1 ml acetone were added to separate litre saline solutions at different temperatures. Bentonite clay (BH-450, <44 μ m) was added to these solutions and allowed to interact with the organic compound under study. Measurement of the amount of hydrocarbon associated with the settled clay showed different degrees of association among the four hydrocarbons investigated and differences due to temperature as presented in Table 1. The data suggest that as a hydrocarbon becomes more soluble with increasing temperature, less of the compound can become associated with clay or other sediment particles.

Table 1 Association of Hydrocarbons with Bentonite Clay in Saline Solutions

Compound	Melting point (°C)	% Uptake*
Normal alkanes:		
Eicosane	38	99% at -1° C 94% at 25° C 83% at 53° C
Hexadecane	20	56% at 25° C
Aromatic hydrocarbons:		
Anthracene	218	60% at -1° C 22% at 25° C 0% at 53° C
Phenanthrene	100	0% at 25° C

* Percentage of hydrocarbon removed from water and found associated with bentonite.

We calculated the heats of sorption of eicosane and anthracene onto bentonite clay using the solution concentrations remaining after clay recovery at different temperatures to determine equilibrium constants for the different hydrocarbon-bentonite associations. The values determined were -2.1 kcalorie mol⁻¹ for eicosane and -9.1 kcalorie mol⁻¹ for anthracene. These heats of sorption indicate physical adsorption of van der Waals type, which is usually less than 10 kcalorie mol⁻¹ (ref. 4).

Sediment samples were obtained from stations in Narragansett Bay⁵ except that Station B, one-half mile south of Station C of ref. 5, was sampled instead of the latter station. Portions of the <44 μ m fraction were treated with 30% hydrogen peroxide under reflux for 48 h to remove indigenous organic matter.

Hydrocarbon association by sediments is summarized in Table 2. These values increase, as does organic carbon, as

Table 2 Hydrocarbon Association with Marine Sediments in Saline Solutions at 25° C

Sediment	% Sediment organic carbon*	Hydrocarbon	Association† μg hydrocarbon/g dry sediment	% Uptake
Station B, total	0.37	Hexadecane	6.1	0.4
<44 μm	0.78	Eicosane	176.0	8.9
<44 μm, H ₂ O ₂	0.21	Eicosane	330.0	17
Station D, total	1.26†	Hexadecane	11.8	0.8
<44 μm	1.46	Eicosane	182.0	9.1
<44 μm, H ₂ O ₂	0.39	Eicosane	435.0	22
Station E ₂ , total	0.98†	Hexadecane	3.1	0.2
<44 μm	1.10	Eicosane	187.0	9.3
<44 μm, H ₂ O ₂	0.30	Eicosane	415.0	21

* Ref. 3. † Ref. 10.

† μg hydrocarbon g⁻¹ dry sediment.

the particle size decreases from total to $<44 \mu\text{m}$. Removal of indigenous organic matter increases the uptake of hydrocarbon by a factor averaging 2.2 for three stations.

Natural sediment organic matter was coated onto a sample of peroxide-treated sediment to ascertain if this material would reduce the uptake of eicosane. The material was obtained from 65 mg samples of $<44 \mu\text{m}$ size Station B sediment by extraction with 1 ml 0.5 N NaOH at 25°C for 72 h, followed by two 2 ml methanol extractions. The extracts were added to 50 mg samples of peroxide-treated Station B sediment ($<44 \mu\text{m}$) and evaporated at 40°C and reduced pressure (100 mm Hg).

The uptake of eicosane was reduced to $146 \mu\text{g g}^{-1}$ sediment, less than half that of the peroxide-treated sediment by this natural organic matter coating (Table 2). The corresponding value for natural sediment is $176 \mu\text{g g}^{-1}$. Because this reduction was brought about by a combination of humic substances and indigenous lipids, a sample of bentonite clay was coated with heptadecanoic acid to determine the effect of a typical lipid on hydrocarbon uptake by sediment minerals. This fatty acid had essentially no effect on the sorption, indicating that indigenous humic substances act alone to interfere with hydrocarbon uptake by sediments.

Table 3 Association of Fuel Oil with Different Minerals in Saline Solutions at 25°C

	Resolved components *		Unresolved components †		Total	
	Weight (μg)	% Uptake	Weight (μg)	% Uptake	Weight (μg)	% Uptake
Initial oil	222.9	—	358.2	—	581.1	—
Bentonite	116.0	52	183.4	51	299.4	51
Kaolinite	65.4	29	96.1	27	161.5	28
Illite	32.7	15	39.1	11	71.8	12
Montmorillonite	8.8	3.9	9.5	2.7	18.3	3.1
Station B, $<44 \mu\text{m}$	17.0	7.6	17.7	4.9	34.7	6.0
Station B, $<44 \mu\text{m}$ (H_2O_2 -treated)	51.5	23	57.4	16	108.9	19
Station B, $<44 \mu\text{m}$ (washed three times)	16.4	7.4	12.9	3.6	29.3	5.0

* Summation of resolved and partially resolved oil components.

† Unresolved complex mixture of oil components. The $<44 \mu\text{m}$ fraction is about 50% illite⁹.

The sorption of No. 2 fuel oil by clay minerals and sediments was investigated in litre saline solutions containing $581 \mu\text{g}$ (approximately $3/4 \mu\text{l}$) of fuel oil added in 1 ml acetone. Analysis of this oil by gas chromatography revealed that it consisted of $222.9 \mu\text{g}$ (38.4% by weight) of resolved and partially resolved components and $358.2 \mu\text{g}$ (61.6%) of a complex mixture of unresolved components. The resolved and partially resolved components (measured as peaks) include n-alkanes, branched alkanes, and isoprenoid hydrocarbons. The unresolved components (measured as a broad envelope) include aromatic and naphthalenic hydrocarbons⁶.

The clay minerals bentonite, kaolinite No. 3, montmorillonite No. 20, and illite No. 35 (all $<44 \mu\text{m}$, ref. 7) were added to the solution. The sorptions by these minerals are presented in Table 3. The resolved and partially resolved components consist mostly of alkanes and would be less water-soluble than the unresolved mixture consisting of more polar aromatic hydrocarbons⁶.

The saline solution from which the bentonite had been recovered was extracted three times with 50 ml portions of chloroform to recover the non-associated fuel oil. The sum of the oil found in the water and on the bentonite was $575.2 \mu\text{g}$, 99.0% of the initial $581.1 \mu\text{g}$ added to the solution.

Samples of the $<44 \mu\text{m}$ fraction of Station B sediment were added to fuel oil solutions, and the oil uptake deter-

mined. Natural sediment and peroxide-treated sediment were used. In addition, a sample of the natural sediment recovered from the oil solution was resuspended three times in fresh 10 ml volumes of oil-free saline solution at 25°C to determine how much associated fuel oil would be washed off (Table 3). Solubility of the petroleum hydrocarbons seems to influence their association with sediment particles in the same manner as found for clay particles.

Three washings of the natural sediment removed $5.4 \mu\text{g}$ of the fuel oil originally sorbed. Most of this loss occurred in the unresolved mixture of oil components. The compounds comprising this mixture are not only relatively water-soluble but commonly include the biologically toxic naphthalenic aromatic hydrocarbons⁶.

The sediment behaved as the laboratory clay experiment predicted, assuming most of the sorption is done by the clay mineral.

The values for hydrocarbon association with peroxide treated sediments in this study were similar to values for hydrocarbon levels in sediments from Narragansett Bay¹⁰. The association of fuel oil with sediments by the method employed in this study seems to be a realistic simulation of the actual process, although dissolved organic matter in seawater may influence natural association¹¹ and cause some deviation from our laboratory values.

Organic matter interferes with oil uptake, perhaps by masking sorption sites in the sediment or by binding sediment particles together, thus reducing the effective surface area.

Three resuspensions of the natural sediment after it had sorbed fuel oil removed only 15% of the oil (Table 3). This suggests that these compounds are firmly associated onto mineral particles in the marine environment, even though only weak physical adsorption seems to be involved in the interaction. Once incorporated into sediments, lipid materials such as fuel oil would not be returned quickly to the overlying water. Because the association forces are weak, however, biological activity and dissolution could slowly release these bound compounds. If dissolution were dominant, then the more polar aromatic hydrocarbons would be released more readily. Due to their relatively high toxicity, these petroleum compounds could impose a continuing stress upon marine organisms long after visual evidence of oil pollution had disappeared.

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Oscillatory Electrochemical Reactions at Bimolecular Lipid Membrane-Redox Electrolyte Interfaces

Pant and Rosenberg¹ have reported sustained, coupled electrical and mechanical oscillations on bimolecular lipid membranes (BLM) separating two aqueous solutions of inorganic salts (Fig. 1). The oscillatory behaviour could be observed in both voltage and current mode. The system showed a definite rectifying characteristic, the transport of negative charge carriers from the compartment containing KI to the $K_3[Fe(CN)_6]$ solution, and/or that of the positive charge carriers from the $K_3[Fe(CN)_6]$ solution to the KI solution, was more likely than that of charge carriers with opposite sign in the same direction.

The characteristic features of the oscillatory phenomena can be summarized as follows. (1) The phenomena are decisively dependent on pH; (2) the oscillations are practically undamped; (3) the oscillations are membrane specific; (4) the amplitude depends on the KI concentration and is independent of pH; (5) the frequency depends sensitively on pH; (6) the oscillations are voltage controlled; (7) the phase difference between electrical and mechanical oscillations is time independent.

Pant and Rosenberg emphasize that the oscillations observed are quite different from those reported by Mueller and Rudin^{2,3} and assumed a similar or identical mechanism to that of Teorell⁴ and Franck⁵. On the basis of more recent investigations⁶⁻¹¹, I now outline theoretical considerations which make it likely that the oscillatory processes described by Pant and Rosenberg are electrochemical electrode reactions (EERs) of electronic nature rather than ionic processes. (Periodic EERs are well known in the classical electronics¹²⁻¹⁶.)

Sustained, undamped oscillations having, in general, a kinetic

$$f(t) = f_0 + \sum_i A_i P_i(t, \omega_i) \quad (1)$$

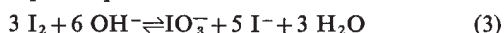
can be expected only in thermodynamically open systems. (Here f_0 is the apparent mean value of $f(t)$; A_i are constants and $P_i(t, \omega_i)$ are periodic functions of cyclic frequency ω_i , with normalized amplitude.) I show that the system meets the requirements of undamped periodic oscillations.

I assume that in the system given in Fig. 1 EERs take place at the interfaces (Fig. 2).

According to the EER

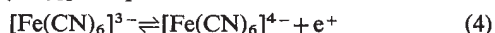


electrons are injected into the membrane at the interface I and the iodine produced partly enters the BLM and partly remains in the aqueous phase. Because of the reaction¹⁶



the iodine remaining in the bathing solution is converted almost completely into iodide (see ref. 17).

In the $K_3[Fe(CN)_6]$ compartment the EER



occurs at the interface II and holes are injected into the membrane. The ferrocyanide ions partly diffuse into the bulk solution and partly link at the interface (reaction (4), in acidic solution, is shifted to the right compared to the equilibrium in neutral solution, and is affected in the same way by illumination¹⁶).

At the same time the reaction



	Aqueous solution		Aqueous solution	
Calomel electrode	0.1 mol l ⁻¹ KCl	BLM	0.1 mol l ⁻¹ KCl	Calomel electrode
	7.4 × 10 ⁻² mol l ⁻¹ KI		1.3 × 10 ⁻³ mol l ⁻¹ K ₃ [Fe(CN) ₆]	
	pH ≈ 10		pH ≈ 5	

Fig. 1 Experimental lipid membrane configuration.

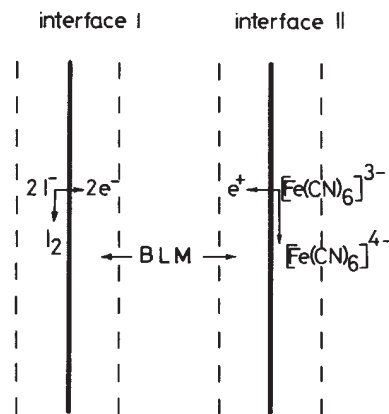


Fig. 2 Movement of ions near the membranes.

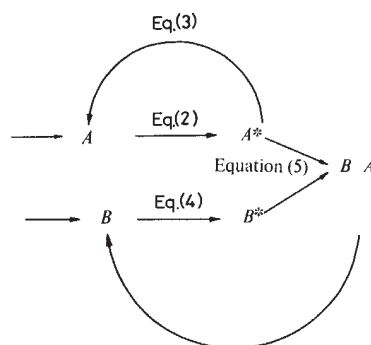


Fig. 3 Overall system of interactions. $A=I^-$; $A^*=I_2$; $B=[Fe(CN)_6]^{3-}$; $B^*=[Fe(CN)_6]^{4-}$.

takes place at the interface II and the reaction products will be partly removed and enter the aqueous phase.

Accepting that these EERs are responsible for the EMF generated and charge carrier injection at the interfaces, the BLM and its immediate environment can be considered as thermodynamically open systems connected by the bulk membrane phase and surrounded by the bathing solutions as reservoirs of infinite capacity.

I now consider, in general, a BLM separating two suitable redox systems A and B (I_2/I^- and $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$). The momentary potential difference (EMF) measured between the bulk aqueous phases is the sum of the single electrode potentials occurring at the interfaces:

$$E = E_0 + A_0 \ln \frac{a_A(ox)^{v_A}}{a_A^*(red)^{v_A^*}} + B_0 \ln \frac{a_B(ox)^{v_B}}{a_B^*(red)^{v_B^*}} \quad (6)$$

where E_0 , A_0 and B_0 are constants determined by the redox systems and temperature, $a_A(ox)$, $a_A^*(red)$ and so on are the activities of the redox components present at the interfaces at any time t ; v_A , v_B and v_A^* , v_B^* are stoichiometric coefficients of the oxidized and reduced species occurring in the stoichiometric equations of the EERs.

Although the single electrode processes are very simple, the system as a whole is complicated (Fig. 3) and the changes in concentrations of the reaction partners with time can be considered only by a nonlinear differential equation system, the general solution to which cannot be given. As can be

seen in Fig. 3 the system considered contains two subsystems which are capable of oscillating, in principle, themselves and are connected (and synchronized) by a diffusion process.

If the activities a_i of the redox components are periodically changing with time at the interfaces ($a_i = a_{i0} + a_{i1} P_i(t)$; $i = A, B$) then the momentary value of the EMF is given by

$$E = \bar{E}_0 + A_0 \cdot \frac{v_A a_{A1}}{A_0 v_A^* a_{A1}^* / a_{A0}^* P_A(t) - B_0 v_B a_{B1} / a_{B0} P_B(t)} - \frac{A_0 v_A^* a_{A1}^* / a_{A0}^* P_A(t) - B_0 v_B^* a_{B1}^* / a_{B0}^* P_B(t)}{A_0 v_A^* a_{A1}^* / a_{A0}^* P_A(t) - B_0 v_B^* a_{B1}^* / a_{B0}^* P_B(t)} \quad (7)$$

so the EMF fluctuates periodically around the stationary value

$$\bar{E}_0 = E_0 + A_0 \ln a_{A0} / a_{A0}^* + B_0 \ln a_{B0} / a_{B0}^* \quad (8)$$

Similar expressions can be derived for the current.

As far as the mechanical oscillations are concerned, one has to assume that there is an energy barrier for the ions generated at interface II, and thus these ions can accumulate at the BLM (Fig. 4; see ref. 17) and the electrostatic repulsion among these bounded ions strives to increase the surface area of the BLM. This increase of surface area occurs to the detriment of the Plateau-Gibbs border and lasts as long as the molecular architecture of the BLM begins to change. The architectural change may lead to increasing separation between polar groups and to a decreasing activation energy for leaving the membrane; consequently the bounded iodide ions can stream into the aqueous phase. After depletion the BLM takes on its original shape and the process starts again.

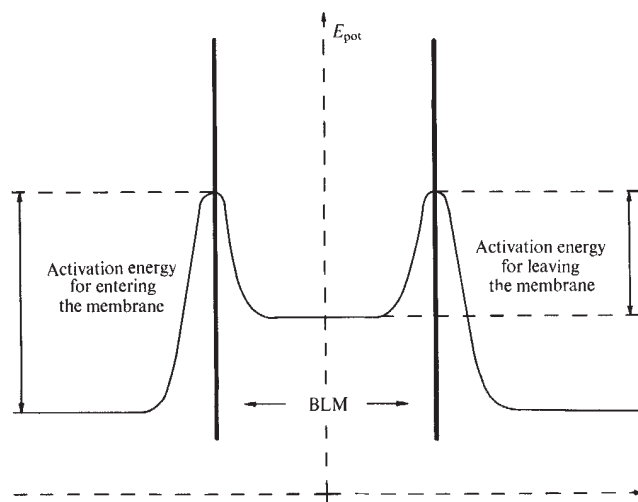


Fig. 4 Energy barrier of the BLM.

Although Pant and Rosenberg found an oscillation of the Plateau-Gibbs border, they did not observe the oscillating bending out of the membrane, in accordance with my assumption. Due to the geometrical circumstances (Pant, personal communication), the BLM behaved like a concave mirror with oscillating curvature. Accordingly, the reflected light beam moved parallel or nearly parallel to the direction of observation and its motion was not observable.

Finally I note that this interpretation supports some suggestions of theoretical and practical importance, namely the iodine-BLM systems are predominantly electronic conductors; the interfaces between the BLM and aqueous phase electronically may behave as semiconductor electrodes; coupled (synchronized) electrochemical electrode reactions occurring at the interfaces are capable of bringing about sustained and temporary processes of regulation and passing of information; electrochemical deposition on and liberation from the membrane surface endow the membrane with dynamic character;

the coupled diffusion and phase boundary effects may play an important role in creating biological oscillations.

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Freely Falling Clocks

SURELY Ellis¹ has not really answered Dingle's question. For the question was: "Which of the two . . ."; not: "Which of all possible . . .". Suppose that neither of the clocks was in free fall.

Incidentally, if that be the answer, am I to suppose that Born was wrong? He answered that Dingle should have asked a different question; presumably that was intended to mean that the original question had no answer.

Also, perhaps I could be told how a theory can deal with free fall, if it does not deal adequately with gravity, which is surely the same thing as free fall considered from another viewpoint. And how can the invocation of free fall provide the solution to a problem in the formulation of which nothing was said about free fall, or any other accelerated motion?

Dingle remarked that everyone who answered his question had a different answer. Maybe the time has come for all of those who want to answer to get together and to come up with one official answer. Otherwise, the plain man, when he hears of this matter, may exercise his right to remark that when the experts disagree they cannot all be right, but they can all be wrong.

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- ¹ Ellis, G. F. R., *Nature*, **242**, 143 (1973).

In Defence of Dingle's Opponents

ARMSTRONG¹ has suggested that if Dingle's work did not deserve serious thought and discussion it might have been better to ignore it completely.

After several letters of his, all of them embodying the same basic mutation of conventional views on relativity, had been answered by various people²⁻⁴, his next two or three letters were indeed neglected. The result of this was a letter from him saying that as nobody seemed to be able to answer his points, he could only take it that everybody now agreed with them.

I continued a desultory private correspondence for some time. I stopped when he answered a particularly detailed analysis which I gave, involving no mathematics worse than simple algebra, showing where I believed his error to lie, by saying simply that it was not his business to find the error in my analysis. As my analysis led to the conclusion with which he disagreed, it must clearly be wrong, and it was up to me to find the error for myself.

His basic error has been to suppose that because it is impossible for one observer to determine whether or not he is moving with respect to another, when each of them is in continuous uniform relative motion, it is therefore impossible to tell which of them has been accelerated. At no time has he been willing to accept that this could make any difference, and at no time therefore has he discussed quantitatively the results of this acceleration.

I do not suggest for an instant that his persistence has had no value. His public persistence has for years made the teaching of relativity more interesting, and has led innumerable students of physics to find a real interest in calculating exactly by how much one of the twins in the paradox could return younger than the other. After doing this calculation themselves they rarely believe that there could be no difference in age.

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Received April 9, 1973.

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² McCrea, W. H., *Nature*, **216**, 122 (1967).

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Another Answer to Dingle's Question

DINGLE¹, in restating his question to avoid the answers of Ziman² and Ellis³, has erred. It is not necessarily true that a time interval measured by two observers in two frames of reference, A and B, will be related by the factor $\gamma(v) = (1 - v^2/c^2)^{-1/2}$ where v is the velocity of one frame relative to the other.

If I define a time interval (Δt) by causing two light flashes, and this interval is measured by an observer in A who is moving from my right to my left with speed u and also by one in B who is moving with the same speed but in the opposite direction, so that the two observers pass each other directly in front of me, then by symmetry, both will record the same time interval between the two events ($\gamma(u)\Delta t$) even though their relative velocity is $v = 2u/(1 + u^2/c^2)$ and even though each observes that the clock in the other frame of reference appears to be running slow.

In this problem I am considering three frames of reference, A and B, and C which is the one in which the two events appear to occur at the same point. In general, no two of these frames will be stationary with respect to one another and their relative velocities will determine whether it is the observer

in A or the one in B who records a longer time interval. There is no ambiguity.

In the particular case when one of the observers is at rest in C it is he who records the shorter time interval. As far as I know, whenever the twin "paradox" is restated to avoid accelerations (by using more than two observers and synchronizing clocks) the "paradox" disappears and all observers agree as to who measures the longer time intervals.

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Received April 11, 1973.

¹ Dingle, H., *Nature*, **242**, 423 (1973).

² Ziman, J., *Nature*, **241**, 143 (1973).

³ Ellis, G. F. R., *Nature*, **242**, 143 (1973).

Whippman's Answer

THE answer to the question posed by Professor Dingle¹ is quite simply that the Lorentz transformation formula does not imply what he claims it does. In the situation he describes, the interval dt' that B's clocks would show is related to the interval dt shown by A's clock by the usual formula

$$dt' = \gamma(dt - \beta D)$$

where β and γ have their usual significance, and D is the coordinate difference between the two events as measured in A's frame. This formula involves only the relative velocity β of A and B, and it is clearly impossible to deduce from it anything about the relative sizes of dt and dt' . The common result, described too succinctly by the phrase "the moving clock appears to be slow", refers only to the very special case where the coordinate difference D is zero. Even in this case, the corresponding difference measured by B will not be zero, the situation is clearly asymmetric and no paradox arises.

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Received April 24, 1973.

¹ Dingle, H., *Nature*, **242**, 423 (1973).

Stedman's Answer

THE feature of the situation for which Dingle¹ is searching is that the two events he considers define a third inertial frame, C say, in which the two events occur at the same position. My one sentence answer to Dingle's question is: the time interval calculated by A is greater (less) than that calculated by B if the relative speed of C and A is greater (less) than the relative speed of C and B.

In connexion with the question which Ziman and Ellis answered¹, and which was suggested by Dingle's earlier, imprecise, formulation of his question, it might be noted that it is sometimes necessary to choose between geodesics which connect the same events².

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¹ Dingle, H., *Nature*, **242**, 423 (1973).

² Holstein, B. R., and Swift, A. R., *Am. J. Phys.*, **40**, 746 (1972).

BIOLOGICAL SCIENCES

Action of Heparin on Nuclei: Solubilization of Chromatin enabling the Isolation of Nuclear Membranes

INTERACTIONS of natural and synthetic polyanions with chromatin are well documented, both at the structural and functional level. Frenster¹ has shown that polyanions can enhance RNA transcription of chromatin, and this has been further studied by Chambon *et al.*^{2,3}. Kraemer and Coffey⁴ have established that morphological modifications such as swelling of nuclei, loosening of the chromatin, and nuclear DNA release correlate with polyanion action⁵. All these phenomena have been logically interpreted as interactions of polyanions with histones leading to the release of genetic restrictions that histones produce, and Miller *et al.*⁶ and Berlowitz *et al.*⁷ have shown that selective binding of histones occurs. Arnold *et al.*⁸ have

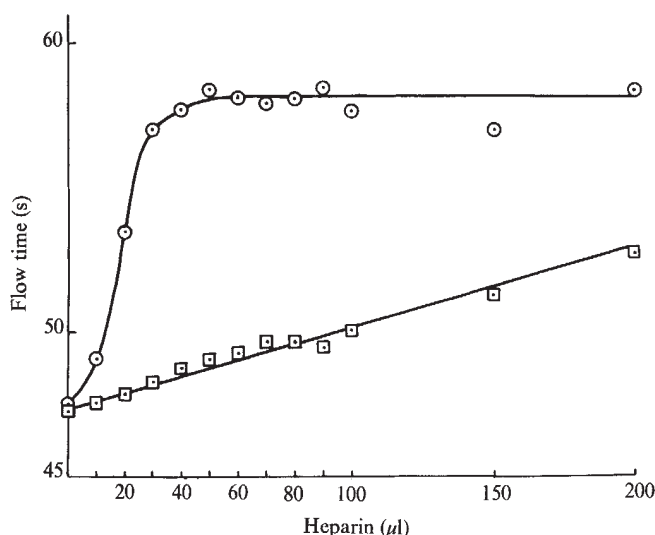


Fig. 1 Viscosimetric titrations. Nuclei were suspended in 0.25 M sucrose by homogenization in a 'Teflon'-glass homogenizer (three strokes); the suspension contained 248 μg DNA ml^{-1} . Aliquots of 1.5 ml were divided into two series; one series received 0.17 ml of 0.020 M Na_2HPO_4 in each tube to give a final concentration of 0.002 M PO_4 . Increasing amounts of heparin (Na^+ salt—grade 1—Sigma): 10 mg ml^{-1} were added in parallel to the two series. After mixing by vortex, 5 ml of water was added to each tube and mixed. All the preceding operations were carried out at 4° C. The tubes were then spun for 15 min at 45,000g (21,000 r.p.m.—rotor JA 21—Beckman-Spinco J21). Supernatants were kept in an ice bath and their viscosity was checked by recording their flow time in a Cannon-Ubbelohde semi-micron dilution viscometer (size 150) maintained at 10° C. Each value is the average of three recordings. □—□, No phosphates; ○—○, 0.002 M Na_2HPO_4 .

made an extensive morphological study of the structural alterations that are produced in nuclei by those polyanions which are capable of removing template restrictions. In all cases, molecular weights of the polyanions seem to be important⁴⁻⁸. As natural nuclear polyanions are known to occur, several theories have been proposed concerning the biological relevance of the above data¹⁻⁹.

Whitfield *et al.*¹⁰ have demonstrated that the complete and lethal dissolution of thymocyte nuclear structure after irradiation, which involves the appearance of free histones¹¹ and free DNA in the cytoplasm¹², is caused by accumulation of inorganic phosphate in the nucleus. On the other hand, Li *et al.*^{13,14} have shown that a low concentration of phosphate induces important and reversible conformational changes of calf thymus histone IV (glycine-arginine rich histone or F_{24}).

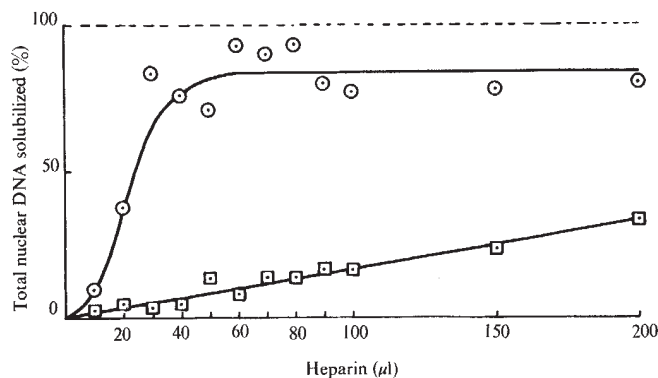


Fig. 2 Solubilization of nuclear DNA in the experiment described in Fig. 1. Aliquots of the supernatants were used to determine DNA. After precipitation by 0.2 N perchloric acid, samples were extracted twice with chloroform-methanol (2/1) and then hydrolysed with 0.3 N KOH at 37° C for 1 h. After acidification, the insoluble products were rinsed twice with 0.1 N perchloric acid. DNA was hydrolysed with 0.5 N perchloric acid at 70° C for 20 min. The hydrolysis was repeated once. DNA was measured by phosphorus determination and by the diphenylamine reaction. Results were in agreement. □—□, No phosphate; ○—○, 0.002 M Na_2HPO_4 .

I have reinvestigated the action of a few polyanions on rat liver nuclei in order to test the effect of phosphate. To monitor the polyanions' action, I assumed that complexing of the histones would release the supercoiled structure of the chromatin that they have been reported to produce^{15,16}; as a result, an increase in the viscosity of the nuclear suspension should be detected.

The results described here are for the action of heparin. Nuclei from rat liver were isolated according to the method of Blobel and Potter¹⁷ and resuspended in appropriate hypotonic medium by rehomogenization with a glass-'Teflon' homogenizer (three strokes).

In a standard experiment, increasing amounts of heparin were added to aliquots of a nuclear suspension maintained in an ice-bath. After mixing, the suspensions were spun for 15 min at 20,000 r.p.m. in a 'Spinco J 21' (45,000g, at R_{max}). Supernatants were diluted with cold water or buffer. Alternatively the dilution could be done before centrifugation, after heparin action. The flow time of each supernatant was measured in a capillary Cannon-Ubbelohde semi-micron dilution viscometer (size 150) maintained at 10° C.

A typical experiment is shown in Fig. 1. The effect of increasing amounts of heparin in the absence of phosphate was a slight and linear increase in the flow times. When Na_2HPO_4

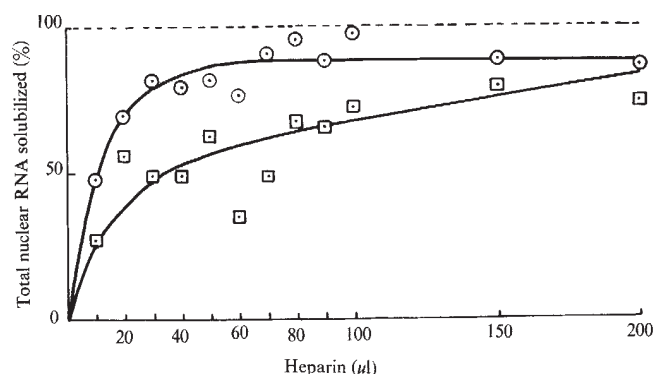


Fig. 3 Solubilization of nuclear RNA in the experiment described in Fig. 1. RNA determinations were performed on the alkaline hydrolysate by absorbance at 260 nm, assuming that 32 mg of RNA per ml would have an absorbance of 1.0 at 260 nm. □—□, No phosphate; ○—○, 0.002 M Na_2HPO_4 .

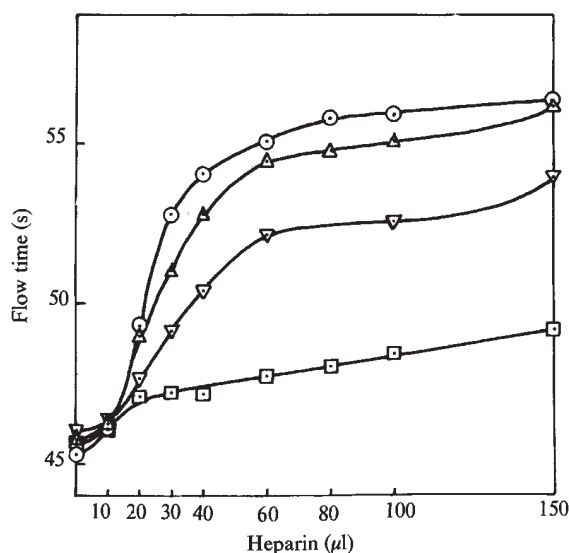


Fig. 4 Viscosimetric titrations showing the cooperative action of heparin. Nuclei were resuspended in 0.002 M Na_2HPO_4 ; the suspension contained 375 μg DNA per ml. Aliquots (1 ml) were divided into four series. The first series was used as such for heparin action. The other three were diluted with 0.002 M Na_2HPO_4 before heparin action: one was diluted once (DNA concentration before heparin action: 187 μg ml $^{-1}$), the second was diluted four times (94 μg DNA ml $^{-1}$), the last one was diluted seven times (55 μg DNA ml $^{-1}$). After heparin action and before centrifugation, all the volumes were made identical by adding 0.002 M Na_2HPO_4 . ○—○, 1 ml of nuclear suspension + heparin + 6 ml of 0.002 M Na_2HPO_4 ; △—△, 1 ml of nuclear suspension + 1 ml of 0.002 M Na_2HPO_4 + heparin + 5 ml of 0.002 M Na_2HPO_4 ; ▽—▽, 1 ml of nuclear suspension + 3 ml of 0.002 M Na_2HPO_4 + heparin + 3 ml of 0.002 M Na_2HPO_4 ; □—□, 1 ml of nuclear suspension + 6 ml of 0.002 M Na_2HPO_4 + heparin.

was present (0.002 M), flow times increased much more, following a sigmoidal curve. A plateau was reached for a ratio of heparin to DNA close to 1, on a weight basis. Fig. 2 shows, for the same experiment, that the flow times recorded correspond to the DNA content of the solutions. Recovery of DNA in the supernatant varied between 85% and 100% at the plateau from one experiment to another. RNA release, shown in Fig. 3, had a different profile; early release seemed to occur either with or without phosphate. With a high concentration of heparin, roughly 90% of RNA was released.

The sigmoidal shape of the viscosity increase suggests that the action of heparin involves a cooperative type of binding. To test this, the same amount of nuclei was suspended in different volumes of 0.002 M Na_2HPO_4 . After heparin action and before centrifugation, all volumes were made identical by adding 0.002 M Na_2HPO_4 . The results are shown in Fig. 4; they clearly establish that cooperation occurs. Some kind of binding involving at least two sites on the same molecule is no longer possible if the concentration of nuclei is too low.

In the standard conditions used, nuclear suspensions in 0.002 M Na_2HPO_4 had a pH close to 8.0. The effect of the pH of the nuclear suspension before heparin action was tested at 7.0, 7.5, 8.0 and 8.5 (0.010 M Tris-HCl buffers), all of them with 0.002 M Na_2HPO_4 . No effect was noticed in this range.

When phosphate was present, the pellets obtained after 15 min at 45,000g were basically nuclear membranes, as observed by electron microscopy (Fig. 5). Very often they resembled purified fraction, which shows the effectiveness of heparin. The general features of the nuclear envelope were present, particularly the pores. There was no nuclear material bound to the membrane, and the outer membrane was covered with ribosomes. Nucleoli were never seen in those pellets. Only thin chromatin threads could be observed. Purification of these nuclear membrane pellets by flotation through a density gradient showed that they were almost pure nuclear

membranes. The bulk of the material floated at a density lower than 1.18 and almost nothing was left at the bottom of the tube.

I think this technique will be very useful because of its simplicity. It is a new method of isolating nuclear membranes. Other known methods involve dilute citric acid¹⁸, hypotonic shock¹⁹, high ionic strength^{20–22}, DNase²³, or DNase plus high ionic strength^{24,25}. A detailed study of nuclear membrane preparations obtained in this way will be published elsewhere.

The ready solubilization of chromatin obtained here is probably caused by specific modifications of some histone binding either to DNA or to other histone molecules. The result is a cooperative modification of the overall tertiary structure of chromatin. When processed for electron microscopic observation (spreading on 0.1 M ammonium acetate, followed by shadowing with platinum), the solubilized chromatin appeared as in Fig. 6—a very complicated network of threads with different diameters, the more abundant having an average diameter of approximately 50 Å. A more complete characterization of this material will be reported elsewhere.

Phosphate-induced conformational changes have been shown with calf thymus histone F_{2a1} (ref. 14). This histone has remained unchanged over billions of years²⁶. On the basis of electrophoretic behaviour, Panyim *et al.*^{27,28} have shown that arginine-rich histones (F_{2a1} and F_3) strongly resist evolutionary change, and are tightly bound to DNA. If they are the target molecules in the heparin action, the effect on rat liver nuclei could be expected to be obtained with any type of nucleus.

What is the biological relevance of these results? Phosphate but also sulphate, and ATP (or GTP) at very low concentra-

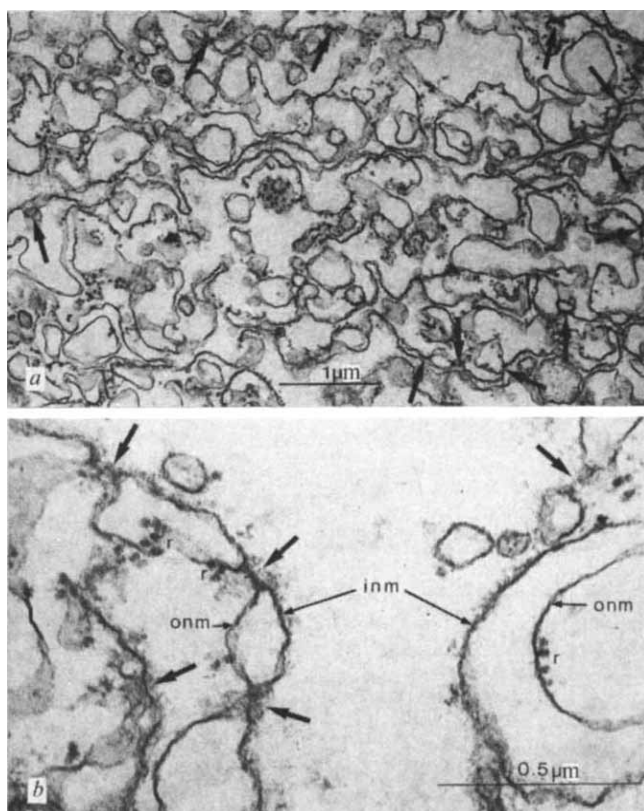


Fig. 5 General appearance of the pellet obtained after heparin action in the presence of phosphate. The rare non-membranous material which could be seen corresponded to thin threads of chromatin. In this experiment, the nuclei were resuspended in 0.25 M sucrose–0.002 M Na_2HPO_4 before heparin action and the heparin to DNA ratio was about 2 on a weight basis. Pellets were fixed with 2% glutaraldehyde and with 2% osmium tetroxide, dehydrated in acetone and embedded in 'Epon'. The ultrathin sections were contrasted with uranyl acetate and lead citrate. Fat arrows, nuclear pores; inm, inner nuclear membrane; onm, outer nuclear membrane; r, ribosomes. a, 4,500×3; b, 18,000×3.

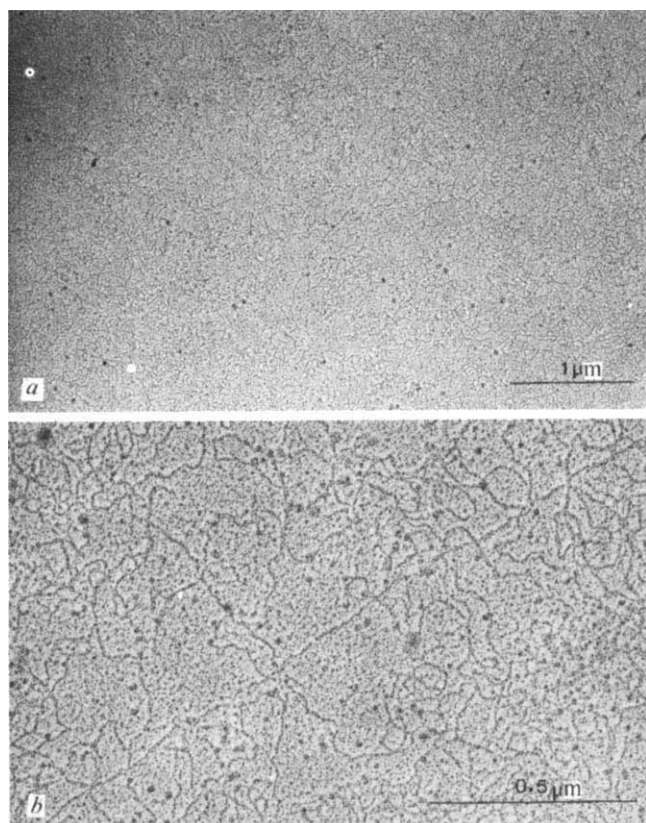


Fig. 6 General appearance of the chromatin solubilized by heparin in the presence of phosphate. The chromatin solution, containing $20 \mu\text{g}$ of DNA ml^{-1} , was spread on the surface of 0.1 M ammonium acetate solution; no cytochrome *c* was added. The film was taken on carbon coated grids which were air dried before shadowing with platinum. *a*, $5,520 \times 3$; *b*, $18,400 \times 3$.

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tions, induce the same conformational changes in F_{2a1} (ref. 29). My colleagues and I are currently testing the effects of these anions on nuclei. It could be that simple conformational changes controlled by the local ionic environment as well as *in vivo* chemical modifications of histones³⁰ are involved in modulating genetic restriction. For example, the nucleolus has a much higher inorganic phosphate content than the rest of the nucleus³¹.

As far as heparin itself is concerned, Kinoshita's results with sea urchin eggs³² indicate that it has an important biological role. It is remarkable that, *in vitro*, heparin can solubilize the genome of a highly differentiated mammalian cell in a well defined organelle, and dissociate it from the nuclear membrane.

I thank Dr B. M. J. Revet for helpful discussions and suggestions in the use of viscosimetric techniques and Dr E. Delain for help with the shadowing techniques.

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Role of Microtubules in Lipoprotein Secretion by the Liver

TRIGLYCERIDE-rich lipoprotein particles, in particular the very low density lipoproteins (VLDL), are important secretory products of the liver¹. In the study of the formation and release of these particles, progress has been made by the demonstration that VLDL can be visualized by electron microscopy as electron-opaque particles, diameter 300–1000 Å (ref. 2). This makes it possible to follow the time sequence of their intracellular migration from their site of formation to that of their release into the extracellular space. Although there is evidence that final discharge of these particles into the space of Disse occurs through fusion of the membrane of the vacuoles which contain lipoproteins, with the plasma membrane³, the mechanisms governing the intracellular translocation of the VLDL particles are still unknown. Circumstantial evidence has led to the suggestion that microtubules and microfilaments are part of a contractile system of the cell which plays an important role in the secretory processes of several different cell types^{4–9}. To investigate the possibility that microtubules are also involved in the secretion of lipoprotein particles by the liver, we made use of the mitotic-spindle inhibitors, vincristine and colchicine, agents which interact with these structures and interfere, probably selectively, with their function^{10–13}. Using this approach, we report here a marked decrease in triglyceride-rich lipoprotein particle release by livers perfused in the presence of mitotic-spindle inhibitors.

Livers from normal albino mice were perfused according to a procedure that we have described elsewhere¹⁴. The perfusion medium consisted of Krebs-Ringer bicarbonate buffer, with

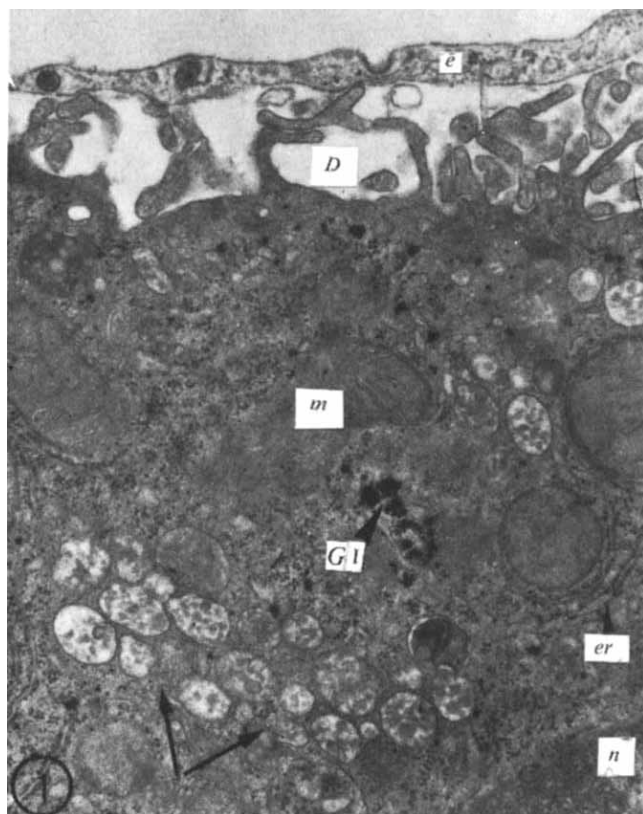


Fig. 1 Vincristine-treated liver. Portion of hepatocyte at the vascular pole. A large collection of vacuoles containing lipoprotein-like particles (arrows) is seen in the juxta-nuclear region. The Disse space (*D*) is devoid of lipoprotein-like particles. *m*, Mitochondria; *er*, rough surfaced cisternae of endoplasmic reticulum; *Gl*, glycogen; *n*, nucleus; *e*, endothelium. ($\times 21,000$.)

25% dialysed bovine serum (charcoal treated), 20% washed bovine red blood cells and 1.5% defatted dialysed bovine albumin to which was then bound 1.5–1.7 mM oleate. The triglyceride output into the perfusate, taken as an overall index of the secretion of triglyceride-rich lipoproteins, was measured by determining fluorometrically the glycerol derived from triglyceride hydrolysis¹⁵. Medium oleate uptake was measured by the method of Ho¹⁶ and ketone body production according to Bergmeyer¹⁵. At the end of the experiment, livers were fixed by perfusing a 2% glutaraldehyde solution at room temperature, then postfixing with OsO_4 , dehydrated with alcohol and embedded in 'Epon'. Thin sections were observed with a Phillips 300 electron microscope.

Table 1 shows that the addition to the perfusion medium of "spindle agents" such as vincristine (2.5×10^{-6} M) or colchicine (10^{-4} M) resulted in a marked decrease in triglyceride production over the 2 h perfusion time used. Table 2 shows that neither the uptake of oleate nor fatty acid oxidation to ketone bodies was affected by these drugs, suggesting that the observed

Table 1 Triglyceride Secretion by Perfused Mouse Livers: Effect of Mitotic Spindle Inhibitors

Addition	Triglyceride output ($\mu\text{mol}/100 \text{ g body weight}$)
0	17.7 ± 1.4
Vincristine	5.9 ± 0.8
0	21.8 ± 1.3
Colchicine	7.3 ± 1.0

Livers from normally fed mice were perfused for 120 min with recirculating medium. Krebs-Ringer bicarbonate buffer containing 25% charcoal treated ox serum, 20% washed ox red blood cells and 1.5% albumin added with 1.5–1.7 mM oleate. Vincristine 2.5×10^{-6} M; colchicine 10^{-4} M. Each figure is the mean of six to eight values $\pm$ s.e.m.

Table 2 Fatty Acid Uptake and Ketogenesis by Perfused Mouse Livers: Lack of Effect of Mitotic Spindle Inhibitors

Addition	Fatty acid uptake ($\mu\text{mol } 100 \text{ g}^{-1} \text{ body weight}$)	Ketogenesis ($\mu\text{mol } 100 \text{ g}^{-1} \text{ body weight}$)
0	124.6 ± 14.1	244.5 ± 39.6
Vincristine	113.2 ± 5.6	209.5 ± 21.2
0	146.7 ± 5.5	259.0 ± 26.4
Colchicine	145.4 ± 2.1	273.1 ± 55.5

Experimental conditions as described in Table 1. Ketogenesis = acetoacetate + β -hydroxybutyrate. Each figure is the mean of six to eight values $\pm$ s.e.m.

decrease in the triglyceride output was not related to altered fatty acid metabolism. Although not shown in this table, it was also found that perfusion of livers with vincristine or colchicine did not modify glucose production, ureogenesis, oxygen consumption or cellular ATP content.

The unlabelled triglyceride content of livers treated with vincristine or colchicine was higher than that of controls (controls: $10.6 \pm 0.9 \text{ mg g}^{-1}$ wet liver, 2.5×10^{-6} M vincristine 13.7 ± 1.7 ; controls 8.8 ± 1.4 , 1×10^{-5} M colchicine 12.9 ± 1.6). Because of the great variability of triglyceride content from animal to animal these differences did not reach statistical significance. Furthermore, because of the small size of a mouse liver, measurement of triglyceride content in each liver, both before and after perfusion, by means of an initial liver biopsy, was impracticable. But labelled oleate incorporation into liver triglycerides was higher in "mitotic-spindle-inhibitor"-treated livers than in controls (for example controls 8.0 ± 0.3 , 1×10^{-5} M colchicine $11.9 \pm 0.6 \text{ d.p.m. g}^{-1}$ wet liver/ $2 \text{ h} \times 10^{-8}$, $P < 0.0025$), thus indicating that triglyceride accumulation was occurring although it was not chemically detectable.

Microtubules have been previously recognized in the cytoplasm of hepatocytes¹⁷. We also observed them by electron microscopy during this study in sections obtained from normal perfused livers. In contrast, microtubules could no longer be found in livers that had been perfused with vincristine. In these conditions, numerous collections of vacuoles containing VLDL-like particles, scattered within the cytoplasm of the hepatocytes, and surrounded by amorphous material were observed at a time when VLDL-like particles were exceedingly rare in the space of Disse (Fig. 1). Occasionally, paracrystalline precipitates were seen in both hepatic and Kupffer cells of livers perfused with vincristine. In hepatocytes, these precipitates were usually associated with the masses of amorphous material surrounding the vacuoles noted above (Fig. 2). After perfusion with colchicine, most liver sections were completely devoid of microtubules although occasional sections showed a few of these structures scattered in the cytoplasm. As in vincristine-treated livers, perfusion with colchicine caused the appearance of many vacuoles containing VLDL-like particles, while the space of Disse was virtually devoid of free lipoprotein particles. Neither vincristine nor colchicine brought about ultrastructural changes in other organelles, and fat droplets occurring free within the cytoplasm appeared to be of the same number in both control and treated livers.

From these experiments it seems that alterations in the microtubular system of the hepatocytes produced by vincristine or colchicine have far-reaching consequences on liver lipid secretion. As fatty acid metabolism is not altered by vincristine or colchicine, and as the only ultrastructural changes appear to be an accumulation of vacuoles containing VLDL-like particles, together with a disorganization of the microtubular system, it is suggested that these drugs produce rather specific effects on microtubular structure and function, thus interfering with the orderly movement of triglyceride-rich lipoprotein particles to the extracellular space. It is not yet known how the blockade of the microtubular system occurs, and how it is related to the accumulation of vacuoles containing

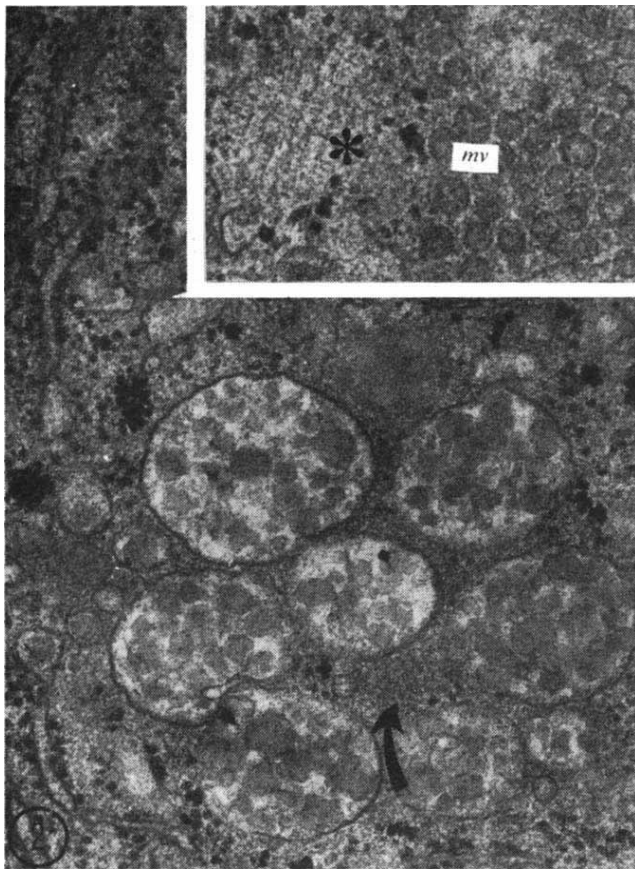


Fig. 2 Vincristine-treated liver. Higher magnification of a collection of vacuoles containing lipoprotein-like particles ($\times 54,000$). Amorphous material can be seen between them (arrow). The insert shows a characteristic paracrystalline precipitate (*) surrounded by a swarm of microvesicles (mv). ($\times 62,000$.)

the VLDL-like particles. Neither is it known whether fatty acid incorporation into lipoprotein triglycerides or amino acid incorporation into lipoprotein proteins are also impaired in livers that have been perfused with the mitotic spindle inhibitors. Experiments are currently being carried out to clarify these points. We would like to propose tentatively, however, that the integrity of the microtubular system is required for the intracellular translocation of triglyceride-rich lipoprotein particles and, therefore, that this system plays an integral role in the sequence of events leading to lipoprotein secretion by the liver.

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Electron-dense Particle in Cholinergic Synaptic Vesicles

SINCE the initial descriptions of synaptic vesicles^{1,2} and their hypothesized relationship with the secretory and electrophysiological phenomena associated with synaptic transmission³, many workers have attempted to distinguish different types of vesicles in different nerve terminals. Known adrenergic terminals contain vesicles approximately 400 and 1000 Å in diameter, both of which may have large dense, granular cores⁴. Similar vesicles of diameter 1400 Å have been described in a mammalian sympathetic interneurone⁵ and are thought to contain noradrenaline⁶. Vesicles of diameter 1400 Å with a large granular core of low electron density have been described in nerve terminals in Auerbach's plexus; these are thought to release ATP on stimulation⁷. Electron micrographs of known cholinergic nerve terminals at mammalian neuromuscular⁸ and torpedine neuroelectroplaque⁹ junctions show large populations of synaptic vesicles having diameters of about 400 and 800 Å respectively but with no evidence of a core structure.

Torpedine electric organs are of increasing research significance, in part because they represent a unique source from which large quantities of an apparently single population of synaptic vesicles can be obtained. We have studied the effect of fixation conditions on the preservation of cholinergic nerve terminals in the main electric organ of the torpedine ray, *Narcine brasiliensis*. We report here that it is possible to prepare the tissue so that a small, electron-dense particle is observed in most of the synaptic vesicles. Using a similar fixation procedure, it is possible to demonstrate the dense particle in isolated vesicles.

The rays were prepared for excision of the electric organ by cooling from 23° C to 4° C in sea water for 1 h in a cold room. They were then pithed, the electric organs were removed and part of the tissue was sliced thinly by hand and immersed in fixative solution for 24 h at 4° C. The remainder of the tissue was frozen in liquid nitrogen for the isolation of synaptic vesicles. The fixation solution was prepared by adjusting the osmolarity of the non-aldehydic constituents to that of elasmobranch body fluids, that is, 870 mosmol l.⁻¹ (ref. 10). The solution contained 4% formaldehyde, 5% glutaraldehyde, 0.09 M CaCl₂ in a 0.3 M sodium cacodylate/HCl buffer, pH 7.2. Diced slices and isolated vesicles were washed after fixation for 4 h in a solution similar to the above except that the aldehydes were absent. Postfixation was carried out in 1.5% osmium tetroxide in 0.1 M sodium cacodylate/HCl buffer containing 0.09 M CaCl₂. The samples were dehydrated in alcohol and embedded in 'Epon-Araldite'. The sections were

examined in a JEM 100B electron microscope with and without uranyl acetate/lead citrate staining.

Isolated vesicles were obtained by following published procedures¹¹ except that, after grinding the frozen tissue, it was extracted with 0.1 M Tris buffer, pH 7.0, containing 0.38 M sucrose, 0.186 M NaCl, 0.014 M KCl, 0.005 M CaCl₂ and 0.005 M MgCl₂. This solution is iso-osmotic with respect to elasmobranch body fluids and has a density approximating the equilibrium density of these vesicles as established in continuous sucrose gradients¹¹. Coarse particles were removed by preliminary centrifugation at 2,000g for 30 min. The extract was then treated with a mixture of apyrase, myokinase and AMP deaminase to destroy free ATP¹² and 5 ml. was layered over 5 ml. of a 0.7 M sucrose solution containing the salts described above. Centrifugation was carried out at 113,500g for 90 min. Acetylcholine was measured by the guinea-pig ileum method¹³; ATP was assayed using the firefly luciferin-luciferase system¹⁴. ATP has recently been shown to be present in isolated cholinergic vesicles of *Torpedo marmorata* electric organ¹². Our study confirms this in *Narcine brasiliensis*. On scanning the distribution of acetylcholine and ATP in 0.6 ml. fractions of the density gradient, we found that 64% of the stable acetylcholine and 63% of the stable ATP showed no tendency to migrate in the centrifugal field. A portion of this material (2.5 ml.) was mixed with an equal volume of the fixative solution described above and the vesicles were pelleted by centrifugation at 114,000g for 120 min. The supernatant was decanted off and replaced by full strength fixative solution for 24 h at 4° C.

As described previously, the electric organ consists of stacks of flat electroplaque cells copiously innervated by nerve terminals lying in shallow troughs^{8,15}. The terminals contain a few mitochondria and numerous synaptic vesicles of diameter approximately 800 Å. In our material, most of the vesicles contained a single, extremely electron-dense particle of diameter about 250 Å (Fig. 1). In some cases, individual vesicles gave the impression of having partially breached the presynaptic membrane (Fig. 1, arrow). Similar dense granules were readily observed in the isolated vesicles (Fig. 2). As the dense particles were clearly apparent in unstained sections, they are either intensely osmiophilic or consist initially of electron-dense material.

Previous workers have reported 300 Å extravesicular granules as well as the 800 Å agranular vesicles in electric organ terminals^{8,15}. These granules may have been released

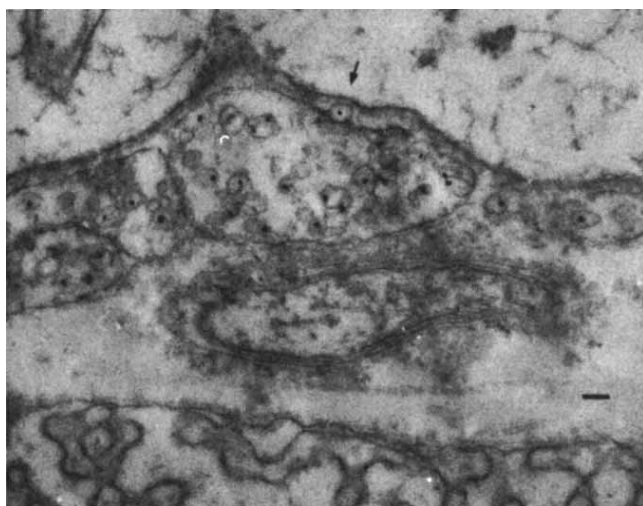


Fig. 1 Nerve terminal lying against the ventral face of an electroplaque cell from the principal electric organ of *N. brasiliensis*. Arrow indicates a vesicle which appears to have breached the presynaptic membrane. Bar length is equivalent to 1000 Å. See text for fixation conditions.

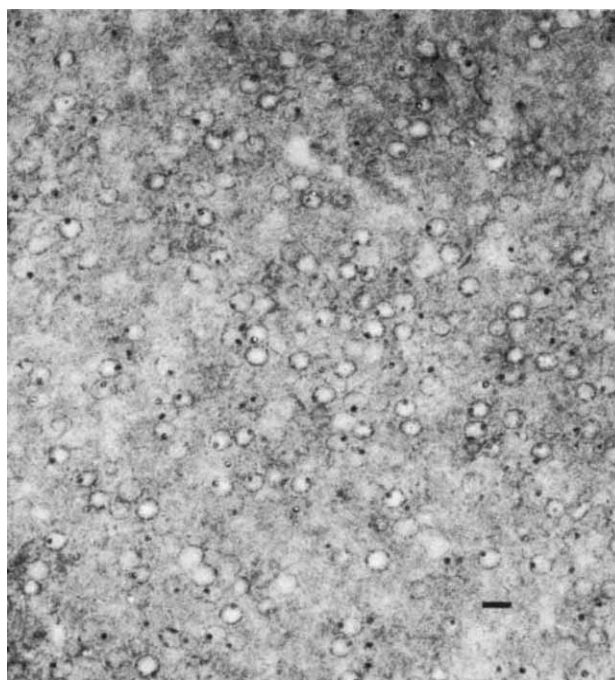


Fig. 2 Isolated cholinergic synaptic vesicles from *N. brasiliensis* main electric organ. Bar length is equivalent to 1000 Å. See text for vesicle isolation and fixation techniques.

from synaptic vesicles during fixation. There have also been reports that certain techniques allowed visualization of dense particles in cholinergic vesicles of frogs¹⁶ and electric eels¹⁷ and of uncharacterized vesicles in mammalian central nervous system¹⁷. These studies involved staining for cholinesterase and it was suggested that the particles were deposits of heavy metals used as trapping agents in the histochemical procedure^{16,17}. A zinc iodide-osmium impregnation technique has also been shown to cause staining of cholinergic synaptic vesicles¹⁸; however, the specificity of this technique is in doubt¹⁹.

The question is, are the cholinergic vesicles of *Narcine* electroplaques intrinsically different from those in mammalian neuromuscular junctions? We are attempting to check the generality and specificity of the observations presented here by using various fixation conditions to prepare the central nervous system of *Narcine* and known cholinergic junctions from other animals. The physiological significance of the results described here is being investigated by stimulating the electric organ to determine (i) the fate of the dense particles and (ii) the incidence of the vesicle/membrane interactions illustrated in Fig. 1.

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Dietary Carbohydrate Increases Brain Tryptophan and Decreases Free Plasma Tryptophan

THE rate at which the brain synthesizes serotonin varies physiologically as a function of its tryptophan concentration^{1,2}. It has been shown that brain tryptophan, in turn, depends on the relative concentrations in plasma or serum of tryptophan and of the other neutral amino acids which compete with it for uptake into the brain³. Thus, carbohydrate ingestion, which raises plasma tryptophan while depressing the concentrations of its competitors, increases the amount of tryptophan in the brain and accelerates synthesis of serotonin in young rats⁴; on the other hand, protein consumption causes proportionately greater increases in the other neutral amino acids than in plasma tryptophan, and thus fails to elevate brain tryptophan or serotonin⁵.

Tryptophan exists in plasma in two forms, namely in a "bound" form, reversibly associated with albumin, and as the "free" amino acid⁶. It has been suggested that the level of free tryptophan in serum determines the concentration of tryptophan in the brain and, consequently, the rate of synthesis of serotonin⁶. We have recently shown that the administration of glucose to human subjects causes a decrease in the quantity of free tryptophan in serum, without affecting the concentration of that bound to albumin⁷. Evidence is presented here that carbohydrate consumption also reduces the amount of free tryptophan in the serum of rats, and at the same time elevates brain tryptophan. Thus the concentration of free tryptophan in the serum does not predict the changes in brain tryptophan that normally accompany such physiological occurrences as eating.

Male Sprague-Dawley rats kept at 20–22° C in a room lit between 0900 and 2100 h by 'Vita-Lite' (50–75 foot candles; Duro-Test Corp., North Bergen, New Jersey) were deprived of food, but not water, for 15–18 h before the start of each experiment. The animals were decapitated and blood collected from the cervical wound into tubes previously flushed with a mixture of 5% CO₂ and 95% N₂ to ensure maintenance of pH. Serum was collected by centrifugation in conditions of minimum haemolysis. The concentration of free tryptophan in serum was estimated by equilibrium dialysis^{7,8}. Visking dialysis tubing (flat width 1 cm, lengths 18 cm) was boiled twice in 0.0002 M disodium EDTA and twice in distilled water, and stored in distilled water. The dialysis buffer was Krebs-Ringer improved bicarbonate, pH 7.45. In the glucose experiment, the buffer also contained small molecular weight constituents of plasma, as recommended by McMenamy *et al.*⁸; however, this addition had no significant effect, and was omitted in later studies. The

tubing was first knotted at one end and 0.5 ml of buffer added. This was followed by a second knot, approximately 6.5 cm from the first, and the dialysis bag was folded in half and put in a tube containing 2 ml of serum. (The same concentrations of free tryptophan were obtained using serum: buffer ratios of 4:1 and 15:1.) After flushing with the CO₂-N₂ mixture, the tube was stoppered tightly. Dialysis was at 37° C in a shaking water bath for 3.5 h to achieve equilibrium⁴. In establishing the method, the levels of free tryptophan were found to be 6.72 µg ml⁻¹ at 2.5 h, 7.2 µg ml⁻¹ at 3.5 h and 7.27 µg ml⁻¹ at 4.5 h. Concentrations of total and free serum tryptophan were determined on aliquots of the serum and buffer, respectively, using the fluorescence assay of Denckla and Dewey⁹. Bound tryptophan was calculated from these concentrations with appropriate volume corrections. Serum NEFA was estimated by the method of Dole and Meinertz¹⁰, and serum tyrosine by that of Waalkes and Udenfriend¹¹. Brains were removed immediately after decapitation, frozen on dry ice, and stored at -70° C until tryptophan was assayed⁹.

In the first experiment, rats were killed 1 or 2 h after being fed a glucose solution (2 g in 4 ml) through a stomach tube. This treatment caused a large increase in the total concentration of tryptophan in serum; this increase was restricted to the albumin-bound fraction while free tryptophan decreased significantly from 5.5 to 4.2 µg ml⁻¹ (Table 1). As in human subjects consuming a glucose load⁷, NEFA fell by almost 50%. Brain tryptophan rose significantly even though free tryptophan in serum declined.

Table 1 Effect of Glucose Ingestion on Serum and Brain Tryptophan

	Control	Glucose (1 h)	Glucose (2 h)
Serum total tryptophan (µg ml ⁻¹)	16.2±0.2	19.6±0.6†	19.9±0.4‡
Serum free tryptophan (µg ml ⁻¹)	5.5±0.1	4.8±0.3*	4.2±0.2‡
Free (% of total)	34	25	21
Serum bound tryptophan (µg ml ⁻¹)	10.7±0.3	14.8±0.6†	15.7±0.5‡
NEFA (meq l ⁻¹)	1.147±0.034	0.648±0.077‡	0.604±0.044‡
Brain tryptophan (µg g ⁻¹)	4.16±0.42	6.42±0.56†	5.93±0.72‡

D-Glucose (2 g 4 ml⁻¹ tap water) was administered to three groups of thirty-five rats (108–146 g) through a stomach tube. Controls received tap water through a stomach tube. Serum was pooled from seven samples; all values are given as mean ± s.e.m.

* $P < 0.05$, differs from controls.

† $P < 0.01$, differs from controls.

‡ $P < 0.001$, differs from controls.

To confirm that the above glucose effect was mediated by insulin secretion, other rats received a dose of exogenous insulin (2 U kg⁻¹, i.p.) previously shown to increase plasma and brain tryptophan¹². This altered the distribution of plasma tryptophan between free and albumin-bound forms in a manner similar to dietary glucose: free tryptophan was 6.1±0.3 and 7.7±0.6 µg ml⁻¹ in hormone-treated and control animals, respectively ($P < 0.05$); serum total tryptophan was 22.2±0.1 and 18.0±1.4 ($P < 0.02$). Confirming previous findings in rats⁴, exogenous insulin increased the total amount of tryptophan in plasma. In humans, total tryptophan does not rise after insulin injection; however, the circulating neutral amino acids which compete with it for entry into the brain fall markedly¹³.

In a second dietary experiment, fasted animals were killed after being allowed to consume one of two protein-free synthetic diets for 2 h. The first diet, used in previous studies in this laboratory^{3,4}, contained both carbohydrate and fat; the second lacked fat. Both diets raised serum total tryptophan, serum

Table 2 Effects of Carbohydrate or Carbohydrate-Fat Diets on Serum and Brain Tryptophan

	Fasted controls	Diets	
		Carbohydrate + fat	Carbohydrate
Serum total tryptophan ($\mu\text{g ml}^{-1}$)	16.5 $\pm$ 0.3	18.4 $\pm$ 0.5†	19.1 $\pm$ 0.4‡
Serum free tryptophan ($\mu\text{g ml}^{-1}$)	6.2 $\pm$ 0.1	5.7 $\pm$ 0.2*	3.4 $\pm$ 0.2‡
Free (% of total)	37	33	18
Serum bound tryptophan ($\mu\text{g ml}^{-1}$)	10.3 $\pm$ 0.4	12.7 $\pm$ 0.7*	15.7 $\pm$ 0.5‡
Serum tyrosine ($\mu\text{g ml}^{-1}$)	19.5 $\pm$ 0.7	11.7 $\pm$ 0.4	14.4 $\pm$ 0.5
NEFA (meq l $^{-1}$)	0.831 $\pm$ 0.021	0.615 $\pm$ 0.029‡	0.301 $\pm$ 0.024‡
Brain tryptophan ($\mu\text{g g}^{-1}$)	2.24 $\pm$ 0.11	3.07 $\pm$ 0.18‡	3.45 $\pm$ 0.19‡

Groups of twenty-two rats weighing 170–200 g were deprived of food but not water at 1400 h and presented with one of the experimental diets at 1030 h the following day. After 2 h, the animals were decapitated and serum and brains taken for assay. Controls had free access to water and were killed during the experiment. Each serum value is obtained from two pooled samples. Each diet contained: dextrose 270 g, sucrose 221 g, dextrin 270 g, Harper's salt mix 40 g, vitamin mix¹⁵ 10 g, and choline 2 g, to which was added 35 g of agar in 1 l of water. The fat diet had an additional 150 g of mazola oil. All values are given as mean $\pm$ s.e.m.

* $P < 0.05$, differs from controls.

† $P < 0.01$, differs from controls.

‡ $P < 0.001$, differs from controls.

albumin-bound tryptophan, and brain tryptophan; both also depressed serum free tryptophan (Table 2) and the concentrations of serum NEFA and of tyrosine, one of the group of neutral amino acids whose omission from the diet has been shown to facilitate the post-prandial increase in brain tryptophan⁸. This correlation supports our previous suggestion that carbohydrates (and insulin) increase the albumin-binding of tryptophan in serum by decreasing the extent of saturation of albumin with free fatty acids, and thus enhancing albumin's affinity for tryptophan⁷. Animals fed diets containing fat showed lesser falls in NEFA and serum free tryptophan than those eating the fat-free diet (Table 2), and in consequence, serum free tryptophan levels differed significantly between groups of rats eating the two diets (5.7 as opposed to 3.4 $\mu\text{g g}^{-1}$, $P < 0.001$). Even so, brain tryptophan levels were not dissimilar (Table 2).

These observations indicate that the concentration of unbound, or free, tryptophan in the serum does not predict the changes in brain tryptophan caused by such physiological inputs as eating. Free tryptophan might be correlated with brain tryptophan after certain treatments such as drug administration¹⁴ or a prolonged fasting period⁶.

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Cell Budding and Fission in Microspores of *Petunia*

CELL budding is a departure from normal cell division which occurs commonly in yeasts. The propagules of many fungi—conidia (for example in the Zygomycetales) and basidiospores (in the Basidiomycetes)—are formed in a similar way¹. Here, I describe the occurrence, *in vivo*, of cell budding and fission in microspores of certain genetic lines of *Petunia hybrida* (Hook) Vilm. These phenomena have not previously been reported for microspores of higher plants, either *in vivo* or in anther culture. Other departures from the normal course of differentiation of the male gametophyte in higher plants are known^{2–5}.

Microsporogenesis in *petunia*⁶ takes place fairly quickly; meiosis is completed in favourable conditions in about 12 h and first pollen mitosis is reached after an additional 24 h. In an attempt to slow down the division process we exposed the plants to cold temperatures for 24 or 48 h, at which time floral buds were collected, smeared in acetocarmine and observed under the microscope.

The cold treatment slowed down the microsporogenesis considerably, enabling the microsporogenetic stages to be clearly recognized. Cell budding and fission in microspores were also observed. I further found that the critical developmental stage at which microspores undergo cell budding or fission is first pollen mitosis. In lines that showed a high rate of cell budding or fission as a result of the cold treatment (13°–8° C), these phenomena were observed also in greenhouse plants, but at a much lower incidence.

Normal microspores which are about to enter mitosis are round and about 25–30 μm in diameter (Fig. 1a). Cells which are about to undergo cell budding swell, become cylindrical, and may reach up to about two–four times the volume of normal microspores. The increase in volume is a consequence of the formation of a large vacuole (Fig. 1b). Next, a second vacuole appears and a nuclear division which results in two equal nuclei takes place (Fig. 1c). At this stage a furrow, distinguished in the cortex, becomes progressively deeper in a centripetal manner. The furrow is distal to the spindle, separating the new vacuole and part of the cytoplasm from the mother cell. The mother cell does not become halved but a minute daughter cell is formed, into which one of the nuclei migrates (Fig. 1d). The minute daughter cell, after the nucleus has passed into it, is pinched off completely from the mother cell (Fig. 1e). In addition to the asymmetrical cell budding as described, I observed a simple cell cleavage in which the microspore is halved; the furrow is formed in the cortex immediately peripheral to the

Fig. 1 Cell budding in petunia microspores. *a*, A normal microspore at first pollen mitosis. *b*, A microspore with large vacuole which is about to undergo cell budding. *c*, Two vacuole stage and two daughter nuclei: the appearance of the furrow distal to the nuclei is shown, *n*=nucleus. *d*, The furrow progresses deeper, centripetally; one nucleus migrates through the narrow passage. *e*, Daughter cell and minute daughter cell after the completion of the budding process.

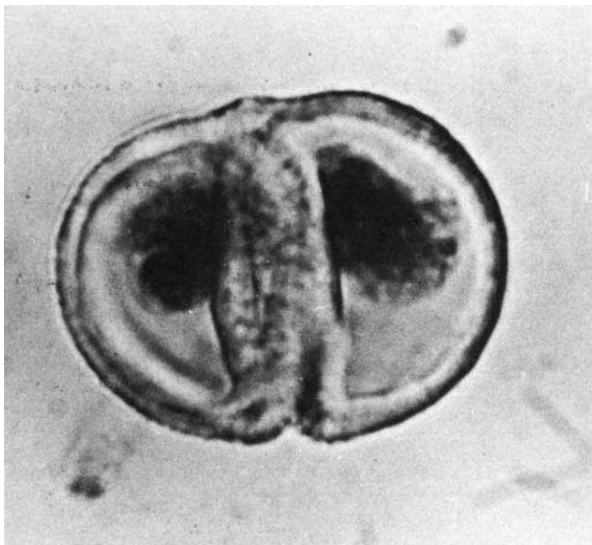
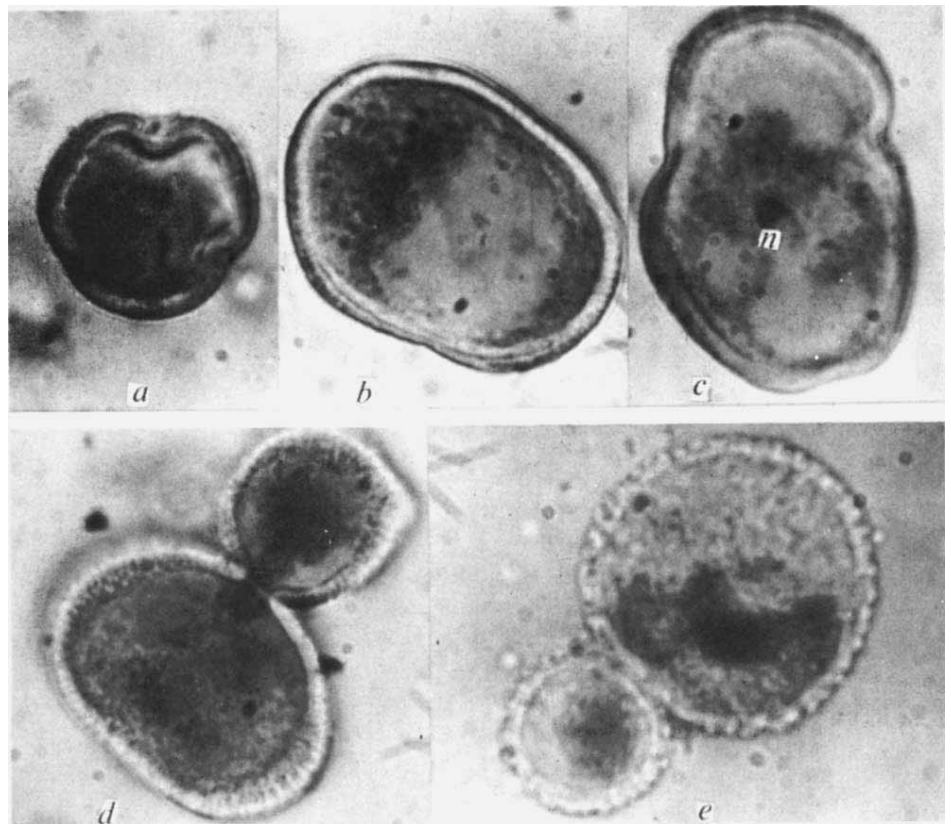


Fig. 2 Cell fission in petunia microspores. Final stage of the cell division process.

equator of the spindle, no nuclei migration takes place, and no cell plate is formed.

Cell fission was observed in the same anthers in which cell budding occurred. Fission progresses according to a normal pattern of simple mitotic division (Fig. 2). I also observed a "bud fission" which is a "compromise" between cell budding and fission (Fig. 3).

These phenomena are clearly departures from the normal course of microsporogenesis. The analogy of the above phenomena in petunia microspores to the reproductive forms of yeasts^{1,7} is striking. It is only in yeasts and petunia microspores that the phenomena are observed in free unicellular structures. That is, the yeasts are unicellular organisms and

the microspores free cells (a true free unicellular stage before pollen mitosis), encased in a pollen sac.

That cell budding and fission in petunia are line specific indicates that these phenomena are under genetic control. The fact that certain genetic lines of higher plants can be obtained that show peculiar traits characteristic of certain microorganisms is of general evolutionary interest. Further, the possibility of inducing divisions in petunia microspores in certain genetic lines may lead to a break through the block of haploid culture of petunia^{5,8}. Further study of the genetic conditions and other aspects of the phenomena described above is in progress.

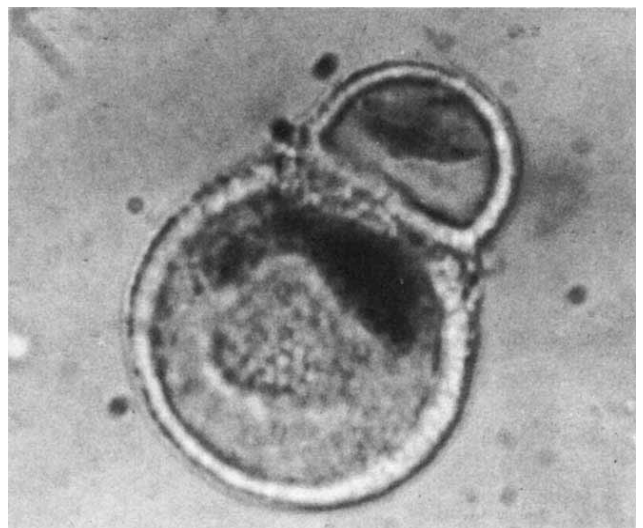


Fig. 3 "Bud fission" in petunia microspores. A furrow is formed but later a cell plate divides between a daughter cell and a minute daughter cell.

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Effects of Light Intensity, Wavelength and Quanta on Gonads and Spleen of the Deer Mouse

LIGHT affects the activity of several endocrine organs of higher vertebrates. In birds the phenomenon usually requires a sufficiently long photoperiod, in some cases the response is intensity-dependent, and in others wavelength appears to be a key factor¹⁻³. The gonads of birds maintained in red light weighed more than those of birds kept in blue or green light of the same intensity⁴⁻¹¹. Not only the pituitary-gonadal axis, but also the adrenal cortex¹², thyroid¹³ and pineal¹⁴ of birds are affected by the photoenvironment.

Only a few reports suggest similar effects of light intensity or wavelength on mammalian endocrine function. Rodents kept in red light reached sexual maturity earlier than those kept in short wavelength light¹⁵. The onset of oestrus in the ferret was dependent on intensity (and possibly wavelength)^{16,17}. Neurosecretory material in the rat hypothalamus was more abundant in animals kept in green light than in those kept in white, red or orange light¹⁸. Enzymatic activity in the rat pineal was most effectively inhibited by green light, less by blue and yellow, and unaffected by red light or ultraviolet¹⁹; the action spectrum was similar to the absorption curve for rhodopsin, suggesting that this retinal pigment mediates the pineal response.

Accurate specification of the physical attributes of light is essential in the design of such experiments. Luminosity units (lumens, lux or footcandles), even when equated in several spectral environments⁴⁻¹¹, describe light in terms of the visual sensation evoked in the average human observer. The receptor mechanism(s) and spectral sensitivities may differ for other vertebrates and/or for non-visual responses. In recognition of this, radiant energy was equated in several recent studies on the action spectrum for the avian photogonadal response²⁰⁻²³.

In spectral environments of equal radiant energy, however, the number of quanta increases with wavelength. Wald²⁴ has therefore suggested that photobiologists should plot spectral functions on a frequency scale, which describes light in direct proportion to quantum energy.

Previous photoendocrine studies have not clearly separated the roles of wavelength, the intensity of radiant energy and number of quanta as they may affect mammalian endocrine function. Here we discuss the relative influence of these three variables on the developing gonads and spleen of the deer mouse, *Peromyscus maniculatus*.

Deer mice were live-trapped during May and June of 1970 and 1971 near Edmonton, Alberta, and maintained in the laboratory with standard mouse diet and water *ad libitum*.

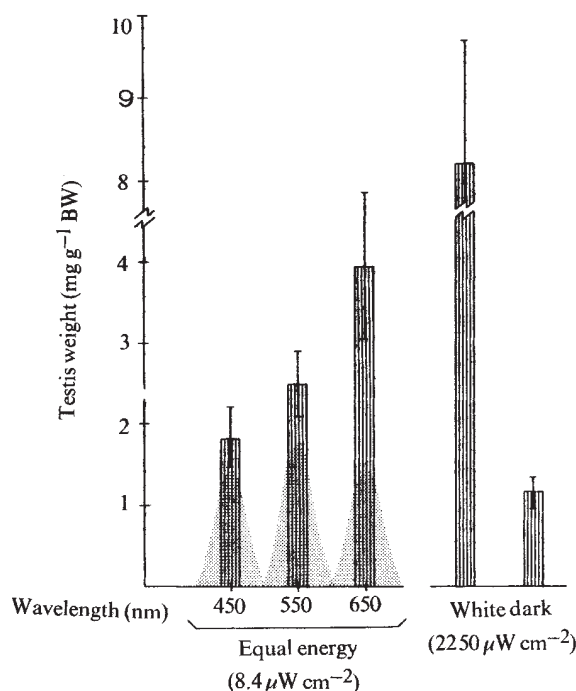


Fig. 1 Testis weight ($\pm$ s.e.) of deer mice kept in several light intensities and in several spectral environments of equivalent energy. As body weight was found to be dependent on light intensity, organ weights are expressed as relative rather than absolute values. Differences are statistically significant at the 0.1% level (2,250 μ W: each of the spectral groups and 2,250 μ W:dark) or 1% level (red:dark) or 5% level (red:blue). Total number for each environment: dark (23); blue (20); green (28); red (22); "bright" white (18); "dim" white (not shown) (17). The shaded grey curves show transmittance of the spectral filters used.

Temperature was maintained at $25 \pm 1.5^\circ$ C. Pregnant females were transferred to plastic mouse cages in chambers designed to provide precise experimental lighting conditions.

The top of each chamber was fitted with a plastic filter measuring approximately 12 $\times$ 12 inches. Coloured filters (CBS filter set, A24631, Carolina Biological Supply Co., Burlington, North Carolina) had peak spectral transmittance of 450 nm (blue), 545 nm (green) or 650 nm (red) (Fig. 1). Light was supplied by a 150 W incandescent floodlamp mounted 20 to 30 inches above the filter. Light intensity was controlled by using neutral density filters made of smoked clear plastic and by adjusting the distance of the lamp from the filter. Cupric sulphate (5% solution, 2 cm deep, in a Pyrex container) served as a heat filter. The intensity of radiant energy measured by a spectroradiometer (ISCO model SR with ISCO model SRC, Instrumentation Specialities Co. Inc., Lincoln, Nebraska) at mouse level inside the chambers is shown in Fig. 1. The number of quanta at peak wavelength for each filter system was calculated according to the formula

$$n = I\lambda/hc$$

where I is the radiant energy at a particular wavelength λ , h is Planck's constant and c is the speed of light.

To determine the effects of intensity and wavelength, forty-seven pregnant females were randomly assigned to six experimental environments; littermates (three to seven) were reared together, from birth to 60 d of age, in 18L/6D. Six litters were reared in "dim" white light of intensity 16 μ W cm⁻²; eight litters were reared in "bright" white light of intensity 2,250 μ W cm⁻²; eight litters were reared in each of three spectral environments of equal energy (8.4 μ W cm⁻²); and nine litters were reared in constant darkness (0L/24D).

To determine the effects of quanta, four litters each were reared in a blue or a red environment with the number of quanta adjusted to be equivalent at peak filter transmittance.

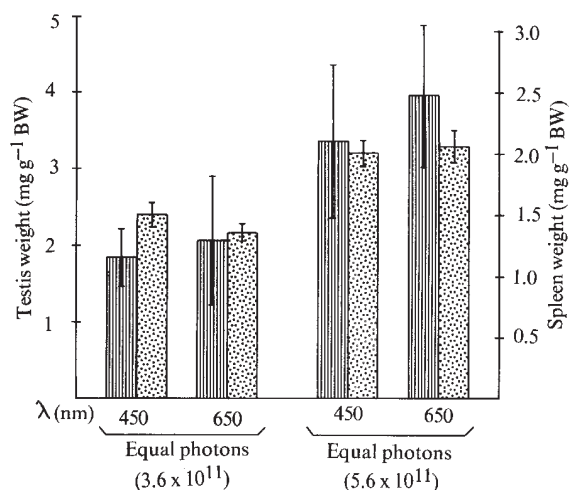


Fig. 2 Testis (hatched columns) and spleen (stippled columns) weight ($\pm$ s.e.) of deer mice in blue and red spectral environments adjusted to provide equivalent numbers of photons at peak wavelength. Organ weight differences within quantum levels are non-significant; the differences between quantum levels are significant at the 5% (testis weight) or 1% (spleen weight) level. Total n for each low photon environment: blue (10♂, 6♀); red (11♂, 10♀); high photon environment: blue (13♂, 13♀); red (12♂, 9♀).

Four more litters were reared in a blue environment with a higher number of quanta, equivalent to that described above.

Data from both experiments were analysed by one-way analysis of variance, and means compared by Duncan's multiple range test as adapted by Kramer for unequal means²⁵.

Body weight of deer mice increased with the intensity of light in which the animals were reared. Testis weight was greatest in animals in the light environment of highest intensity (8.19 ± 1.52 mg g⁻¹ body weight in $2,250 \mu\text{W cm}^{-2}$ as against 4.25 ± 0.68 in $16 \mu\text{W cm}^{-2}$). In spectral environments of equal energy the response was greatest in red light, intermediate in green, and least in blue light. Testis weight of mice in darkness was less than in any other group (1.16 ± 0.19) (Fig. 1).

Ovary weight was greatest in animals reared in bright light and significantly higher in both dim (17.81 ± 3.92) and bright (21.99 ± 3.64) light than in the dark (6.62 ± 0.48). There was no difference in ovary weight between mice reared in the three spectral environments. Histological examination revealed primary and maturing follicles in the ovaries of mice reared in darkness, in dim light and in the three spectral environments. Only in the ovaries of mice reared under bright light could corpora lutea be found.

Spleen weight of mice reared in dim or bright white light, or in red light, was significantly greater than that of animals reared in blue light, in green light, or in darkness.

The different response of testes and spleen to wavelength of light was not observed when the number of quanta at peak wavelength was adjusted to be equivalent in blue and red environments, whether the quantum level was 3.6×10^{11} or 5.6×10^{11} . Testis and spleen weights were greater in environments with the larger number of quanta at peak wavelength, irrespective of colour (Fig. 2).

Growth rate, spleen and gonad weight of deer mice were thus found to be dependent on light intensity. When spectral environments provided equal radiant energy, the testes and spleen developed more rapidly in blue light, irrespective of body weight. When spectral environments were adjusted to provide equivalent numbers of photons at peak filter transmittance, this differential responsiveness to wavelength was no longer observed. These results establish the photoresponsiveness of some components of the maturing endocrine and immune systems of the deer mouse, and suggest that this influence of light depends on the number of quanta rather than on wavelength or radiant energy *per se*.

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Experimental Arthritis in Thymectomized Rats with an Impaired Humoral Immune Response

ADJUVANT-induced arthritis in rats shares many of the clinical and pathological features of human rheumatoid arthritis¹. Cell-mediated immune mechanisms are believed to be of importance in the experimentally induced arthritis². Although this experimental disease can be transferred by thoracic duct lymphocytes from sensitized to non-sensitized syngeneic recipient rats³, it has been reported that neonatal thymectomy does not prevent subsequent induction of adjuvant arthritis⁴. We recently observed induction of adjuvant arthritis in rats subjected to neonatal thymectomy, but not in sham thymectomized rats. The accompanying finding that the severity of the arthritis was related to depression of an apparently thymus dependent antibody response may be a clue to the immunopathological mechanism involved in this arthritis model.

In experiments designed primarily to assess the role of thymus-derived (T) lymphocytes in the pathogenesis of experimental autoimmune encephalomyelitis (EAE)⁵, forty-five NTX Lewis rats, of both sexes and aged 12–14 weeks, and nine sham operated control litter mates, received in two hind foot pad injections a total of 75 μg of syngeneic basic protein of myelin in 50 μl of saline emulsified with 50 μl of complete Freund's adjuvant (CFA, butyricum, Difco Laboratories, Detroit) supplemented with 2 mg of human mycobacteria per ml (Commonwealth Serum Laboratories,

Melbourne). As additional adjuvant, a simultaneous injection of 50 μ l of *Bordetella pertussis* vaccine (special product of Commonwealth Serum Laboratories) containing 10^{10} organisms was given subcutaneously into the dorsum of each hind foot.

Table 1 Incidence of Arthritis in NTX and Sham Thymectomized Rats Inoculated with Adjuvants with and without Basic Protein of Myelin

Inoculum	NTX	Control *
BP/CFA/P	23/45	0/9
CFA/P	3/4	0/1
CFA	3/4	0/1
Total	29/53	0/11

BP, Syngeneic basic protein; CFA, complete Freund's adjuvant; P, pertussis vaccine.

* Arthritis was never observed in previous experiments involving similar inoculation of 278 non-thymectomized rats.

The rats were examined daily for 28 d for signs of EAE. Only one of forty-five NTX rats developed clinical EAE, compared with five of nine controls. Unexpectedly, however, from the sixteenth day after injection, signs of arthritis became apparent in twenty-three of the forty-five NTX rats while none of the sham operated rats developed arthritis (Table 1). In order to examine any possible relationship of arthritis to the later documented immune status of the rats, the arthritis was graded clinically on the basis of daily observations and a score 0 to +++ was assigned to each rat (see Table 2). Early signs of arthritis, before gross swelling of the joints became obvious, superficially resembled mild EAE, but neurological disease was excluded because the tails remained strong and there was no urinary or faecal incontinence. Nodular deformities characteristic of adjuvant arthritis developed in many of the NTX rats' tails and bilateral corneal opacities became apparent in one rat on the twenty-first day. Histological sections of joints from three severely arthritic rats showed proliferation of synovial cells with granulomata and infiltration of lymphocytes and plasma cells (Fig. 1). Giant cells and polymorphs were conspicuous in some areas. Some joint spaces contained inspissated synovial fluid with polymorphs and fibrin. Joints



Fig. 1 Section of an arthritic tarsal joint from a NTX rat (stained with H and E). Proliferation of connective and synovial tissue is marked and there is extensive infiltration with mononuclear cells.

Table 2 Relationship of Low Numbers of Blood Lymphocytes Before Immunization in NTX Rats to Subsequent Poor Antibody Responses and Severe Arthritis

Lymphocytes mm ⁻³ of blood	Microgrammes ¹²⁵ I-basic protein bound per ml of serum	Severity of arthritis *
NTX		
2,200	<0.1	++
2,300	<0.1	++
2,700	<0.1	0
2,800	<0.1	0
3,000	<0.1	++
4,200	<0.1	+++
4,600	3.7	0
4,700	<0.1	0
5,000	4.5	+
5,000	7.4	0
7,000	2.2	0
7,900	<0.1	0
11,000	5.2	0
Control		
6,900	1.8	0
7,400	2.5	0
9,200	0.8	0
10,000	1.8	0

The peripheral blood lymphocyte counts of both NTX and control rats are arranged in ascending order.

* Grading of severity: +++, immobile with gross polyarthritis; ++, visible swelling of joints, movement severely limited by pain with or without tail or eye lesions; +, erythematous swelling of hind feet only; 0, no abnormality noted.

from three NTX rats and one control were cultured for bacteria. No organisms were isolated from the control or from one NTX; *Staphylococcus aureus* grew from the other two NTX joints, but no *Mycoplasma* organisms were isolated. All the rat sera were negative for human rheumatoid factor (Hyland rapid slide test).

The "T lymphocyte status" of randomly selected rats in both NTX and sham thymectomized groups was assessed by the following criteria: preimmunization blood lymphocyte counts, responsiveness of spleen cells to phytohaemagglutinin (PHA) and histological appearance of "thymus-dependent" areas of the spleens. Details of these indices are published elsewhere⁵; however, the values of all these T cell indices were significantly lower in the NTX than in the control group. The three NTX rats with the severest grade of arthritis (+++) were depleted of T lymphocytes by all criteria; the twenty rats with less severe arthritis (+-++) had varying degrees of T cell diminution. Severe arthritis occurred amongst NTX rats which had low blood lymphocyte counts documented before inoculation (Table 2). All available sera from the rats were tested for antibody to myelin basic protein by gel filtration radioimmunoassay⁶. It was found that production of antibody to myelin basic protein was a thymus-dependent response in rats⁵. The serum of NTX rats with a preimmunization blood lymphocyte count $\leq 4,200$ mm⁻³ had no detectable antibody (Table 2) and the overall severity of arthritis was related inversely to the serum concentration of antibody to ¹²⁵I-basic protein (Table 3).

To rule out the possibility that the basic protein of myelin had some role in the induction of arthritis, ten additional rats were injected with adjuvants without basic protein; four NTX and one sham thymectomized rat received CFA and pertussis and four NTX and one sham thymectomized rat

Table 3 Relationship in NTX Rats of Severity of Arthritis to Antigen-binding Capacity of Serum

Severity*	No. of NTX rats	Binding of ^{125}I -basic protein by serum ($\mu\text{g ml}^{-1}$)	
		Undetectable	Detectable (mean $\pm$ s.e.m.)
++ + + +	16	12	4 (0.9 ± 0.3)
+	4	1	3 (2.3 ± 1.2)
0	18	10	8 (2.7 ± 0.9)

* See Table 2.

received CFA alone. Six of the NTX rats, but neither control, developed arthritis of severity comparable with those which received basic protein (Table 1).

These data suggest that T lymphocytes (and associated cell-mediated immunity) are not solely responsible for the pathogenesis of adjuvant-induced arthritis. Thus the following mechanism is suggested to explain the high incidence of arthritis in thymectomized animals. With reduced numbers of T lymphocytes, the decreased capacity to produce antibody to certain "thymus-dependent" antigens may lead to the formation of complexes of antigen and antibody with antigen in excess; such complexes are unsuitable for elimination from the circulation and furthermore are capable of mediating immune damage to tissues (reviewed in ref. 7). An extraordinarily high incidence of rheumatoid arthritis and other "collagen diseases" has been reported in patients with agammaglobulinaemia^{8,9}. Furthermore, Williams *et al.*¹⁰ recently reported that in patients with rheumatoid arthritis, low values for peripheral blood T lymphocytes were significantly correlated with the severity of clinical disease. Further investigation of the function of T lymphocytes in patients with rheumatoid arthritis is indicated. The findings in this study engender caution in the consideration of thoracic duct drainage as a therapeutic measure for patients with rheumatoid arthritis who are refractory to conventional therapy¹¹.

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Mechanism of Host Specificity in Malarial Infection

INDIVIDUAL species of malaria parasites are highly host specific¹ but the mechanisms which determine susceptibility or innate resistance are poorly understood². *In vitro* methods for the short-term culture of erythrocytic forms of *Plasmodium knowlesi*³ and for isolating free merozoite preparations minimally contaminated with host cells⁴ have provided a basis for studying the ability of serum and of red cells from susceptible and resistant hosts to support the growth and multiplication of this simian parasite.

Plasmodium knowlesi parasites⁵ were maintained by weekly passage in rhesus monkeys. Cultures were initiated with infected rhesus red cells and were performed as previously described⁵, but using Medium 199 (Burroughs-Wellcome) free of leucine and supplemented with glucose (200 mg ml⁻¹), penicillin (5 U ml⁻¹) and ATP (0.01 mg ml⁻¹). Parasite growth was assessed by microscopical examination of stained smears and by measuring incorporation of ³H-leucine into parasite protein⁶. The various mammalian sera listed in Table 1 were absorbed with one third of their volume of washed rhesus erythrocytes for 1 h at 37° C followed by 18 h at 4° C. These absorbed sera were used at a final concentration of 10% in the medium and all supported parasite multiplication in 24 h cultures of *P. knowlesi* (Table 1). The multiplication rate was less than three-fold in mouse and pig sera, four to eight-fold in Douroucouli monkey, rabbit, guinea-pig and human sera and about ten-fold in hamster and in rhesus and Kra monkey sera. ³H-leucine incorporation data indicated that the second cycle of *in vitro* development which follows schizogony progressed in media containing sera from all susceptible and most resistant species.

Another series of experiments tested the growth of *P. knowlesi* in red cells from various species. Cultures were initiated with a small volume (10 μl . per flask) of schizont-infected rhesus red cells separated by centrifugation of infected blood at 300g for 8 min, and contaminated with less than 1% of uninfected red cells. Normal erythrocytes from different animals (Table 1) were washed twice with medium and 0.25 ml. of packed cells from each was added to duplicate culture flasks. Rhesus serum, absorbed as described above with the corresponding species of erythrocytes, was used in the medium at a final concentration of 10%. Multiplication of parasites occurred with erythrocytes from all susceptible species (Table 1). Some parasite invasion of red cells from the resistant *Galago* (bush baby) occurred after schizogony (Table 1, 10–12 h) but these parasites did not survive during further culture and were not infective when inoculated into the donor. No parasite invasion was detected with red cells from the remaining five resistant species (Table 1).

Further experiments were designed to test whether susceptibility could be correlated with the presence of erythrocyte surface receptors, which specifically bind free merozoites. Suspensions of schizont-infected cells (0.05 ml. packed cells) containing less than 10% of uninfected rhesus red cells were washed and incubated with 3 ml. volumes of medium. When free merozoites began to appear, samples (0.25 ml.) containing approximately 10⁷ schizonts were incubated with 2 ml. of medium containing about 2 $\times$ 10⁸ washed erythrocytes from various species. These suspensions contained less than 1% of uninfected rhesus erythrocytes derived from the first schizont culture and were examined as follows.

(1) Small volumes were studied by phase-contrast microscopy on a warm stage at 37° C. Merozoites newly released from schizonts and showing some motility were observed for attachment to red cells and, as multiple attachments may lead to lysis of erythrocytes⁷, the incidence of lysed cells was also noted. With rhesus and *M. fascicularis* monkey red cell suspensions 21–38% of free merozoites became

Table 1 *In vitro* Multiplication of *P. knowlesi* Parasites Cultured with the Serum or Red Cells from Various Susceptible and Resistant Species. Results are Mean Values from at Least Two Cultures

Species	<i>In vivo</i> response	Serum × rate (24 h)	× rate (8–10 h)	<i>In vitro</i> response Red cells		× rate (24 h)
				Differential count (8–10 h) % R*	% S*	
Rhesus monkey (<i>Macaca mulatta</i>)	Susceptible	10.3	7.6	99	1	7.6
Kra monkey (<i>Macaca fascicularis</i>)§		9.6	6.9	99	1	6.9
Human		7.6	2.9	99	1	3.7
Douroucouli monkey (<i>Aotus trivirgatus</i>)		4.6	0.9†	92	8	1.1
Bush baby (<i>Galago</i>)	Resistant	—	0.4‡	8	92	0.03
Hamster		9.8	0.5	0	100	0
Rabbit		7.6	0.3	0	100	0
Guinea-pig		7.4	0.4	0	100	0
Mouse		2.5	0.1	0	100	0
Pig		2.5	0.1	0	100	0
Rat		—	—	0	100	0

* R, immature ring forms derived from reinvasion after schizogony; S, schizonts.

† Produced a patent infection when inoculated into the Douroucouli donor.

‡ Did not produce a patent infection when inoculated into the bush baby donor.

§ The natural host for *P. knowlesi*.

attached and 0.5–4% of red cells were lysed. With red cells from rat, rabbit and guinea-pig 1–4% of merozoites became attached to red cells and up to 0.3% of erythrocytes underwent lysis (Table 2.)

Table 2 Microscopical Observations of Merozoite-Erythrocyte Suspensions

Experiment	Erythrocyte species	Merozoites		Red blood cells	
		No. observed	% attached to RBC	No. observed	% lysed
260	Rhesus monkey	89	21	700	4
	Rat	103	1	890	0.3
274	Kra monkey	28	22	212	0.5
	Rabbit	24	4	224	0
277	Rhesus monkey	37	38	338	1.8
	Guinea-pig	78	0	300	0

(2) When merozoite release from bursting schizonts was judged to be maximal, 0.25 mg of phytohaemagglutinin (Burroughs-Wellcome reagent grade) was added to each culture flask containing 3 ml. of medium to agglutinate normal and parasitized red cells; this treatment does not aggregate free merozoites (unpublished results of G. H. M.). After further incubation for 20 min, the aggregated cells were removed by centrifugation at 250g for 5 min. Almost all free merozoites remained in the supernatant after this treatment (unpublished results of G. H. M.) and were counted in a haemocytometer chamber. As shown in Table 3, 30–80% of merozoites were coprecipitated with the red cells from susceptible hosts and 2–10% of merozoites with the erythrocytes from resistant hosts.

Table 3 Attachments of Free Merozoites to Red Cells from Various Species

Erythrocyte species	% total merozoites* bound to RBC
Kra monkey	82
Rhesus monkey	51
Human	29
Mouse	10
Rabbit	8
Guinea-pig	2

Mean values from two or more experiments.

* Total merozoites were estimated from control tubes containing the schizont-infected cells and less than 1% of unparasitized red blood cells.

Plasmodium knowlesi is able to infect a wide range of monkeys as well as the gibbon and man, but lower animals are completely resistant. The *in vitro* experiments described

here show that the properties of serum cannot be correlated with susceptibility or resistance of the donor species. By contrast, humoral factors do have a role in the innate immunity to several other protozoal infections including trypanosomiasis, trichomonas and some avian malarial⁸.

In this study resistance and susceptibility could be correlated with properties of host erythrocytes. Cells from Old World monkeys were more susceptible than those from New World monkeys while erythrocytes from the non-primate species were completely resistant. The erythrocyte requirements for parasite survival seem to be, first, the presence of red cell receptors for merozoites. Parasites are specifically orientated during attachment and always adhere at the conoid end^{7,9}. Red cells from susceptible but not resistant hosts bind merozoites (Tables 2 and 3) and this process appears analogous to the specific tissue binding described for several viruses¹⁰. The nature of the merozoite receptor is unknown but may be related to antigenic properties of red cells¹¹; the infectivity of avian red cells was reported to be unaffected by treatment with trypsin, chymotrypsin or neuraminidase¹².

The second requirement is the presence of a suitable intracellular environment¹¹. In our study this is illustrated by the fact that some invasion of bush-baby red cells did occur (Table 1), but these intracellular parasites failed to survive in culture or after inoculation into the erythrocyte donor.

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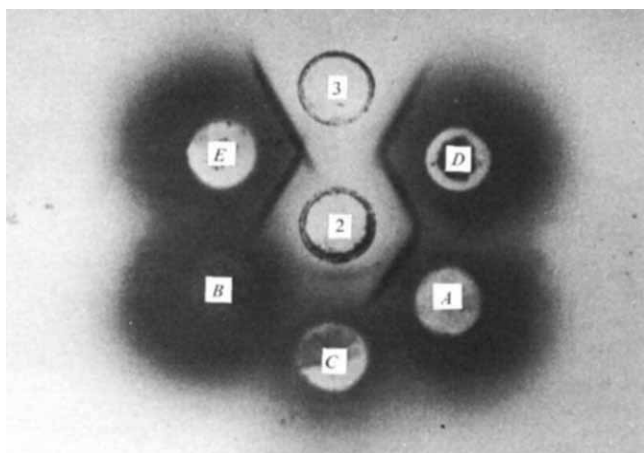


Fig. 3 Autoradiograph of double diffusion plates. Antisera: 2, rabbit anti-mouse κ chains and; 3, rabbit anti(rat)210. Radioactive products of clones prepared as described in Fig. 1. A, P1Bu1; B, 210.RCY3.Ag1; C, P1Bu2; D, hybrid clone HyIII-1; E, hybrid clone HyIII-2.

while ten spreads of the hybrid clone HyIII-1 had counts of 84, 84, 87, 77, 82, 84, 85, 82, 87 chromosomes after 6 months in culture. Only the hybrid lines can grow in the selective medium.

Figure 1 shows a comparison of the proteins synthesized by the different cell lines. In addition to the clones shown in Fig. 1 we have tested eighteen more. All synthesize immunoglobulin molecules of both parental types. One of them (HyIII-19) did not synthesize heavy chains (sample *k*, Fig. 1). The relative intensity of the different bands in the patterns is quite reproducible for each clone, but it differs from clone to clone. For example, clone HyIII-14 (sample *j*, Fig. 1) showed very little free mouse light chain. An important difference between parental and hybrid products is that hybrid cells produce an extra component (X) not seen in either parent. This component is not observed when the proteins produced by the mixture of the two parental lines are subjected to the same procedure (sample *f*, Fig. 1). Component X contains antigenic determinants recognized by both anti-mouse κ chains and anti-210 (rat) antibodies as shown by immunoelectrophoretic focusing studies (Fig. 2). The isoelectric point of X is intermediate between rat and mouse light chains and the band disappears if the material is reduced and carboxamidomethylated before isoelectric focusing. It seems likely, therefore, that it is a hybrid mouse-rat light chain dimer.

The region of the isoelectric focusing plate which contains the IgG molecules shows that they contain rat light chain determinants as well as mouse light chains. Although the anti-rat antiserum cross reacts with free mouse light chains it does not react with mouse light chains in IgG. Molecules made of mouse heavy chain and rat light chain are produced in good yields (Fig. 2). This results in shifts in the position of the major IgG bands (for example, HyIII-14 and 16 in Fig. 1). The occurrence of non-symmetrical molecules made up of one light chain of each parental type is indicated by the results shown in Fig. 3. These show that most of the IgG molecules are precipitated by both anti-mouse and anti-rat light chain antibodies.

Apart from component X which, as discussed above, is likely to be a dimer of one rat and one mouse light chain, no other band observed in the hybrids is absent from both parents. Scrambled molecules, such as would be expected if the integration of V and C sections took place at the mRNA level, were not observed. The possibility that band X contains such molecules is not excluded although it seems unlikely. One would have expected two new molecules banding in different positions and not necessarily recognizing both antisera. It seems, therefore, that the genes for the Ig

chains of both parents are expressed in the hybrid clones. The presence of Ig hybrid molecules indicates that the expression of the two sets of genes is not attributable to the individual expression of segregants of the clones but that both parental genes are expressed in the same individual cell. This implies, therefore, that in a mouse-rat hybrid cell the expression of one light chain is not hindered by the expression of the other. It remains to be established that this is also true of hybrid cells of homologous species. The simplest explanation seems to be that the restricted expression of V and C genes is caused by the presence in the parental cells of single already integrated V-C genes. Such integration could be the result of a translocation event taking place in plasma cell precursors. A single translocation in a non-committed cell could start a chain of events leading to the activation of the integrated gene but not of other non-integrated genes for similar chains—including the sets in the other haploid chromosome. The integrated gene would in this way be active independently of the presence or absence of other integrated similar or homologous genes.

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Why is the XX Fitter? Evidence Consistent with an Effect of X-heterosis in Women from Sex Ratio Data in Offspring of First Cousin Marriages

ALTHOUGH women are known to be more viable than men, the biological mechanisms for this difference are poorly understood. A relevant question is, to what extent does heterozygosity for X-linked loci contribute to survival of the XX

Table 1 Livebirths, Foetal Loss and Death, by Marriage Subset*

		Number of families		Livebirths		Stillbirths			Abor-tions		Acci-dental deaths		Natural deaths		Deaths of un-known cause		Twin sets	
		Total	Fertile	M	F	M	F	?	Nat.	Th.	M	F	M	F	M	F	M	F
X-Outbred Type 1	Rural koseki	96	92	232½†	275	2½†	0	2	11	1	12	2	49	52	3	8	1½	2
	Rural non-koseki	18	12	21	24	1	1	0	0	0	0	0	6	7	0	1	0	0
	Urban non-koseki	13	13	22	26	0	0	0	3	5	0	0	3	6	0	0	0	0
	Totals	127	117	275½†	325	3½†	1	2	14	6	12	0	58	65	3	9	1	2
X-Outbred Type 2	Rural koseki	113	107	255	273	3	3	5	8	16	6	0	61	49	0	1	1	2§
	Rural non-koseki	14	8	15	12	1	0	0	0	0	0	0	4	1	0	0	0	0
	Urban non-koseki	3	2	2	1	0	0	0	0	0	1	0	1	0	0	0	0	0
	Totals	130	117	272	286	4	3	5	8	16	7	0	66	50	0	1	1	2
X-Inbred Type 3	Rural koseki	111	104	290	258	6	2	2	5	3	8	2	63½†	58	8	5	1	1
	Rural non-koseki	12	9	19	20	0	0	0	1	0	0	0	8	6	0	1	0	0
	Urban non-koseki	14	12	22	20	0	0	1	1	0	0	0	3	0	1	1	0	0
	Totals	137	125	331	298	6	2	3	7	3	8	2	74½†	64	9	7	1	1
X-Inbred Type 4	Rural koseki	143	129	340	330	6	3	2	17	6	7	0	71	69½†	2	0	1	1
	Rural non-koseki	19	15	41	21	0	0	2	1	3	3	0	10	10	0	0	0	0
	Urban non-koseki	9	7	24	12	0	1	0	0	2	1	0	4	1	0	0	0	0
	Totals	171	151	405	363	6	4	4	18	11	11	0	85	80½†	2	0	1	1
X-Inbred all	Totals	308	276	736	661	12	6	7	25	14	19	2	159½†	144½†	11	7	2	2
X-Outbred all	Totals	257	234	547½†	611	7½†	4	7	22	22	19	2	124	115	3	10	2	4

* Pedigree and other data were collected in 1964 through a census directed at all marriages, legal or consensual, represented by at least one spouse alive and residing in Hirado at the time of the census⁶⁻⁷. Some 10,530 unions contracted subsequent to 1890 were ascertained, and their reproductive histories recorded. The information obtained at interview was routinely compared with the existing records of the public health office, the agricultural and fishing cooperatives, the tax office, and the kosekika, the office of custody of the koseki, the household censuses required by law in Japan⁸. All vital events which affect the composition of a family such as marriages, births and deaths must be reported to this office. Where there was a discrepancy between the interview and the koseki, the data from the latter were generally used. As reporting of livebirths is very complete whereas most abortions and many stillbirths go unrecorded, the observations here reported on abortions and stillbirths stem almost exclusively from interviews, whereas the livebirth data are a synthesis of interviews and koseki observations and are much more complete. Information on type of death was that obtained by interview. Most of the natural deaths were in early childhood years. About 20% of the deaths recorded were to those over 21 yr of age. Deaths of unknown cause were those which were recorded in the koseki but not recalled by those being interviewed. Almost all the deaths in this category were of individuals who had died in infancy. All twin pairs were concordant for sex, and in view of the very low rate of dizygotic twinning in Japan, each such set of twins was scored as one birth of that sex. One set of triplets resulted in two stillborn females and one liveborn male and was scored as one male livebirth and one female stillbirth (Type 2-Outbred). The following abbreviations are used: M, male; F, female; Nat., natural abortion; Th., therapeutic abortion. There was one child of unknown sex in the rural koseki group of Type 3 inbred marriage, and one natural death to a child of unspecified sex in the rural non-koseki group of Type 4 inbred marriage.

† Where one of the twins was liveborn, the other stillborn or only one died, the entry "½" is made in appropriate column (see above for rationale). ‡ This twin pair included one liveborn and one stillborn. § Both members of one twin set were stillborn.

female? The advantage accruing to the XX female through having this factor can be defined as X-heterosis¹. Practically all serious X-linked disorders such as haemophilia occur in males, but the total incidence of such severe diseases is relatively small and cannot account for the observed sex differences in age specific mortality rates. It is conceivable that X-heterosis is relatively insignificant in the total population, and that the bulk of the observed sex difference is attributable to physiological factors that are a consequence of sex differentiation (for example, the apparent oestrogen-sparing effect on coronary heart disease) and/or to psycho-cultural factors that may pertain to diminished exposure of women to environmental hazards (for example, industrial pollutants).

Large sex differences in foetal death rates are not as well established as sex differences in postnatal death rates. Even if the rates of foetal loss of males and females were identical, however, a significant X-heterotic effect could exist in XX foetuses but be balanced by relatively beneficial physiological factors primarily affecting male foetuses.

X-heterozygosity may not necessarily be beneficial. Evidence

suggests that heterozygosity at the Xg^a blood group locus in one population at least has a deleterious effect during gestation in view of the selective loss of heterozygous female foetuses of Xg^a negative mothers⁹. As yet, there is no evidence for a large beneficial effect of X-heterozygosity in any population for any trait, and there are no data for an analysis of the net total effect of X-heterosis on differential survival in any population.

We present here data which suggest that, among the population of the Japanese island of Hirado, X-heterosis contributes quite considerably to female survival during gestation. There is also a trend consistent with an X-heterotic effect on postnatal survival.

The theoretical basis for the approach used has been described in detail¹ and the discussion is limited here to first cousin offspring (see Fig. 1). Briefly, of the four types of first cousin marriages, two, types 1 and 2, may be said to be "X-outbred" as they result in XX daughters who are X-outbred and two, types 3 and 4, "X-inbred" as they result in XX daughters who are X-inbred. If X-heterosis is significant during gestation then a higher sex ratio at birth (that is of males to

Table 2 Livebirth Sex Ratio and Death Rates by Cousin Subset

	Livebirth sex ratio	Non-accidental death rate*	
		Male	Female
X-Outbred			
Type 1	0.848	0.221	0.228
Type 2	0.951	0.243	0.178
All	0.896	0.232	0.205
X-Inbred			
Type 3	1.111	0.252	0.238
Type 4	1.116	0.215	0.222
All	1.113	0.232	0.229

* The non-accidental death rate is the sum of the natural deaths and deaths of "unknown cause" (which were almost all in the infant years) divided by the number of livebirths.

Table 3 Socioeconomic Status and Maternal Age at Birth, First Child by First Cousin Subset

	Socioeconomic status		Maternal age at birth, first child	
	Mean	Variance	Mean	Variance
X-Outbred				
Type 1	16.73	36.33	21.60	8.38
Type 2	18.28	86.98	22.22	11.61
X-Inbred				
Type 3	16.82	39.43	21.61	15.46
Type 4	17.36	71.43	21.92	13.82

* Socioeconomic status is represented here by a score which is the sum of the coded values associated with parental occupation, education, and related variables. See ref. 7 for details.

Table 4 Distribution of Families by Decade of Marriage

	-1899	1900-1909	1910-1919	1920-1929	1930-1939	1940-1949	1950-1959	1960-
X-Outbred Type 1 (127)	2.4%	8.7%	13.4%	18.1%	10.2%	30.0%	14.2%	3.1%
X-Outbred Type 2 (130)	0.8%	3.8%	14.6%	17.0%	13.8%	28.5%	16.9%	4.6%
Total X-Outbred (257)	1.6%	6.2%	14.0%	17.5%	12.1%	29.2%	15.6%	3.9%
X-Inbred Type 3 (136)	1.5%	8.1%	14.0%	22.8%	13.2%	26.5%	11.8%	2.2%
X-Inbred Type 4 (171)	2.9%	2.9%	18.7%	17.5%	16.4%	26.3%	14.6%	0.6%
Total X-Inbred (307)	2.3%	5.2%	16.6%	19.9%	15.0%	26.4%	13.4%	1.3%

females) would be expected in the infants of X-inbred subsets of first cousin marriages than in those of the X-outbred subsets. And if X-heterosis is significant postnatally then X-outbred females should be relatively protected by this effect compared with X-inbred females. The rationale for these considerations is presented in Fig. 1.

The data presented here were obtained from a large investigation of consanguineous marriages on the island of Hirado (Table 1). There were 565 unions of first cousins for which data were available; this was the largest category of consanguineous matings encountered and the only one on which a detailed analysis was undertaken. The sex ratios of livebirths, stillbirths, and postnatal deaths by first cousin subset appear in Table 1 along with details on the ascertainment and classification of the data.

The secondary sex ratios in both X-inbred subsets are higher

than in both X-outbred subsets (Table 2) and the difference between the sex ratios of X-inbred subsets and X-outbred subsets is statistically significant ($547.5/611 = 0.896$ against $736/661 = 1.113$, $\chi^2 = 7.24$, $d.f. = 1$, $P < 0.01$). If attention is restricted to the most homogenous groups within a subset, the rural, koseki checked pedigrees, the same effect is present ($487.5/548$ against $630/588$, $\chi^2 = 4.65$, $d.f. = 1$, $P < 0.05$). The differences are thus consistent with a negative effect of X-inbreeding during gestation, which leads to a net loss of females in the X-inbred subsets. There is, however, no striking difference in foetal loss between X-inbred and X-outbred subsets. Although even late foetal wastage was probably very poorly ascertained, the lack of any difference here suggests that if X-heterozygosity is responsible for the sex ratio difference at birth, females lost because of X-inbreeding may be lost early in gestation, perhaps before awareness of pregnancy, and reproductive compensation readily occurs in view of the lack of any significant effect on total fertility.

The apparent magnitude of the effect is relatively large, and it may be specific to the population under investigation. In addition, it is present in spite of any influence polymorphism at the Xg^a locus might have had on sex ratio in those studied; but we have no data on the frequency of such alleles at this locus on the island. We also noted that the observed sex ratio in the X-outbred group is not necessarily what would be predicted in a completely outbred control population, as differences between the sexes in response to autosomal inbreeding in both X-inbred and X-outbred subsets may also have had an effect on the sex ratios in those studied here.

Another trend of interest is the postnatal death rate. If X-heterosis is significant for postnatal survival then X-inbred

Table 5 Distribution of Families by Religion

	Buddhist	Catholic	Kakure*	Other
X-Outbred -1 (127)	81.1%	0.8%	17.3%	0.8%
X-Outbred -2 (130)	80.0%	0%	16.2%	3.8%
X-Outbred total (257)	80.5%	0.4%	16.7%	2.3%
X-Inbred -3 (137)	79.6%	0.7%	16.1%	0.7%
X-Inbred -4 (171)	83.3%	0.9%	12.3%	3.5%
X-Inbred total (308)	81.7%	1.1%	14.3%	2.9%

* A syncretic cult incorporating elements of Buddhism and Catholicism.

Table 6 Distribution of Families by Geographic Area

	Hirado -1	Hirado -2	Nakano	Himosashi	Shishi	Nakatsura	Tsuyoshi	Shijiki
X-Outbred Type 1 (127)	9.4%	25.2%	13.4%	10.2%	20.5%	7.1%	6.3%	7.9%
X-Outbred Type 2 (130)	9.2%	17.7%	9.2%	22.3%	18.5%	10.0%	7.7%	5.4%
Total X-outbred (257)	9.3%	21.4%	11.3%	16.3%	19.5%	8.6%	7.0%	6.6%
X-Inbred Type 3 (137)	10.9%	22.6%	15.3%	12.4%	16.1%	10.9%	8.0%	10.9%
X-Inbred Type 4 (171)	9.9%	17.5%	7.6%	18.1%	15.1%	7.0%	12.3%	12.3%
Total X-inbred (307)	10.4%	19.8%	11.0%	15.6%	15.6%	5.5%	10.4%	11.7%

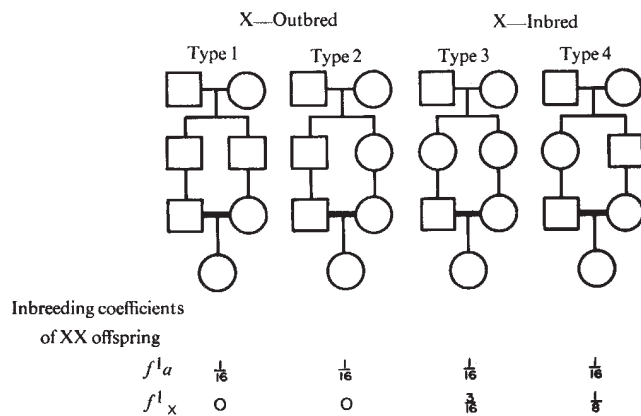


Fig. 1 Possible pedigrees of XX offspring of first cousin marriages. Note that XXs born to first cousins have identical inbreeding coefficients but may have different X chromosome inbreeding coefficients depending on the parental relationship. If heterozygosity for X-linked loci has an effect on a particular attribute, say, survival, this effect should be less for X-inbred females (that is, those for whom $f^I_x > 0$) who will be more homozygous, on the average, than females for whom $f^I_x = 0$, that is, X-outbred females. If X-heterozygosity is beneficial, this advantage attributable to outbreeding should by definition be more significant for X-outbred than X-inbred females but would have no effect on XY males. Therefore, if X-heterozygosity contributes positively to survival to age n , the sex ratios (M/F) of X-outbred groups (that is, those in which X-outbred females occur, Types 1 and 2 above) should be less than those of the X-inbred groups (those in which X-inbred females occur, Types 3 and 4) and conversely if there is a negative effect. The secondary sex ratios, that is those noted at birth, should reflect the net effect of X-heterozygosity during gestation. The occurrence of XO females and males with more than one X chromosome will confound the theoretical expectation, but the instances of such individuals in all populations studied to date appear to be less than 0.2% and are thus likely to be of trivial magnitude. See refs 1, 3 and 4 for further discussion.

females should have greater mortality and morbidity than X-outbred females, but there should be no effect on the males. What data are available are consistent with this hypothesis. Although deaths have probably been poorly ascertained because of migration and other factors, most of those recorded represent deaths in infancy and early childhood. The mortality rates for non-accidental deaths (as defined in Table 2) in males in the two groups are identical, 0.232. But the recorded death rate in X-inbred females, 0.229, is about 12% greater than that in X-outbred females, 0.205. With the number of individuals involved, however, the trend is not yet significant at the 5% level.

Most studies to date of offspring of consanguineous unions have compared them with outbred individuals, and conclusions may always be vitiated by possible systematic differences between the related and unrelated families. Our study, by making comparisons within the inbred group of first cousin offspring, avoids many of these pitfalls. It is still possible, however, that some systematic differences between the subsets might have contributed to the differences in sex ratios. Comparisons of X-inbred groups and X-outbred groups with regard to a number of possibly relevant factors are presented in Tables 3–6. There appear to be no major differences with regard to decade of marriage, geographical distribution on the island, maternal age at birth, socioeconomic status, or religion. This does not exclude the possibility of some other as yet unknown differences between these subsets that might be associated with altered sex ratio. At present, however, the only known systematic difference between these groups is the X-inbreeding coefficients of their female members, suggesting that this is the causal factor for the sex ratio data.

These observations provide the first evidence consistent with a significant deleterious effect of X-inbreeding in any mammalian population.

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Photoperiodism and Adaptive Behaviour in a Small Mammal

SEASONAL changes in photoperiod have wide-ranging effects on the biology of most temperate zone vertebrates^{1,2}. Although photoperiodic responses have been extensively studied in birds³, research on photoperiodic effects in mammals has been confined largely to their influence on reproductive and pelage cycles^{4,5}. Less well understood are the effects of seasonal differences in photoperiod on mammalian behaviour, although modification of behaviour is the principal means by which small mammals adjust to seasonal changes in their environment⁶. Furthermore, seasonal changes in photoperiod may be used as a cue by small mammals to alter behaviour before more critical changes in other environmental conditions, such as temperature. We describe here the effects of previous exposure to differences in both photoperiod and temperature on the expression of nesting, hoarding, daily activity, and food consumption in the white-footed mouse, *Peromyscus leucopus*.

Forty-eight male *P. leucopus* were individually caged and assigned at random to one of the following treatments: (1) warm acclimated (26° C) under a long-day photoperiod (16:8 LD); (2) warm acclimated under a short-day photoperiod (9:15 LD); (3) cold acclimated (5° C) under a long-day photoperiod; or (4) cold acclimated under a short-day photoperiod. (All mice were 4–5 months old and reared in the laboratory on a 16:8 LD photoperiod at 26° C.) After 6 weeks of this treatment, individual mice were placed in a freshly cleaned 10 gallon aquarium for assessment of hoarding behaviour and daily activity. The aquarium was provided with a water bottle and a half pint, blackened jar which was used by the mouse as a harbourage. For three consecutive days, 40 g of soy beans was scattered on the floor of the aquarium, removed the following day, and the amount weighed. Daily activity was measured using an ultrasonic motion detector (Alton Electronics, Gainesville, Florida), which activated a 10 channel Esterline-Angus event recorder. Transmitting and receiving probes were centred and mounted 18 inches apart above the aquarium. After estimation of

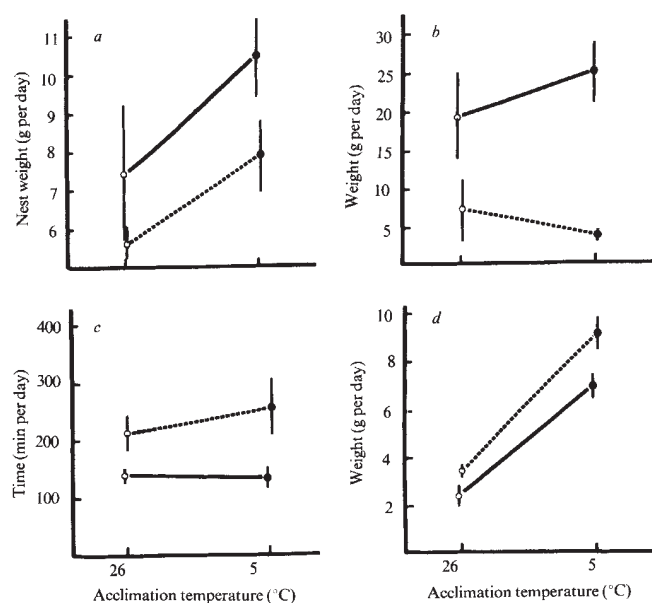


Fig. 1 Mean values ($\pm$ s.e.) for nesting behaviour (a), hoarding behaviour (b), duration of activity (c), and food consumption (d) for forty-eight male *Peromyscus leucopus*. Mice were simultaneously exposed for 6 weeks to either 26°C (○) or 5°C (●) and either long (---) or short (—) photoperiod.

hoarding and activity, nest building was indexed by providing individually caged mice with a preweighed amount of cotton in the food hopper of the cage lid and determining the amount remaining each day for 4 consecutive days. Newly constructed nests were removed each day. Finally, daily food consumption was assessed for 3 d. Because of the possible confounding effect of a new environment, no first day scores were included in the results.

Data were considered to represent a 2×2 factorial arrangement of treatments and subjected to least-squares analysis of variance to determine significance of effects of differences in photoperiod, temperature and interaction of these treatments on the measured behaviours.

Comparison of means indicates that photoperiod is of major importance in altering the expression of nesting (Fig. 1a), hoarding (Fig. 1b), daily activity (Fig. 1c), and food consumption (Fig. 1d). Mice exposed to short-day photoperiod for 6 weeks built larger nests (9.0 against 6.8 g; $t=2.0$; $d.f.=44$), hoarded greater quantities of food (22.4 against 5.5 g; $t=4.6$; $d.f.=44$), were less active (139 min against 235 min; $t=2.8$; $d.f.=44$), and consumed less food (4.7 g against 6.3 g; $t=3.2$; $d.f.=44$) than animals under a long-day photoperiod, irrespective of acclimation temperature. (Although the photoperiodic effect on nest building approaches significance at the 0.05 level of probability, greater differences have been reported for a similar experiment⁷.) Thus, although mice under short photoperiod were less active overall than animals exposed to long photoperiod, periods of activity were directed more toward behaviours associated with thermoregulation and winter survival. These data also demonstrate that differences in photoperiod have manifold effects on the behaviour of this species and suggest that seasonal decreases in photoperiod may serve to mobilize the behaviour of natural populations of *P. leucopus* towards emphasis on winter survival.

In a more general sense, the extent of these photoperiodic effects on behaviour demonstrates the necessity for rigorous control of photoperiod during behavioural research on small mammals.

Mean nest building and food consumption were substantially increased in cold exposed mice relative to warm acclimated animals (9.2 g against 6.7 g; $t=2.3$; $d.f.=44$, and 8.0 g against 2.9 g; $t=10.4$; $d.f.=44$, respectively), regardless of photoperiodic effects; this demonstrates independent

influences of temperature and photoperiod on nesting and food consumption (there was no significant interaction effect between these environmental cues). Temperature differences had little effect on expression of hoarding behaviour and daily activity in this species.

In addition, assessment of all variables on the same animals enabled estimation of correlations between behaviours. Examination of correlations, pooled within treatment groups, reveals that mice which hoarded more food weighed less ($r=-0.47$; $P<0.01$) and also built larger nests ($r=0.29$; $P<0.05$); as might be expected, more active animals consumed more food ($r=0.46$; $P<0.01$). Such correlations provide initial insight into the behavioural composition of this species; but these observed associations alone do not permit distinction between the relative contribution of similar environmental influences and a common genetic basis (usually due to pleiotropy). The latter, referred to as a "genetic correlation"⁸, is of particular interest, as it may reflect how natural selection has moulded mammalian behaviour into a functional system. Experiments are in progress to determine the relative influence of environment and genes on associations between these behaviours.

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Visual Pigment of the Giant Panda *Ailuropoda melanoleuca*

THE death of the giant panda ('Chi-Chi') at London Zoo on July 22, 1972, provided an opportunity for examining the visual pigment of this rare species (*Ailuropoda melanoleuca* David, 1869). An eye was removed as soon as possible after death, and kept in darkness in a freezer. The following observations were made in Sussex between July 25 and 27.

After thawing the eye (18 mm diameter), the retina was removed in red light and washed in neutral buffer solution. The outer segments of the photoreceptors were separated by Saito's method¹ and then extracted at room temperature overnight (17 h) with 0.5 ml of 3% digitonin solution. To improve the spectrophotometric properties of the extract, it was left to stand at room temperature in darkness for 24 h, made 0.02 M in neutral hydroxylamine, and centrifuged. The absorbance spectrum of the clarified extract is given by curve *a* in Fig. 1.

To test its homogeneity, the extract was bleached in stages by successive exposures to lights from a monochromator. After each exposure, the absorbance spectrum was measured (Cambridge SP 500): curve *b* in Fig. 1 was obtained after 55 min of 620-nm light; curve *c* after 60 min of 620-nm light of higher intensity; curves *e* and *d* after 30 and 60 min of 600-nm light respectively. In all these exposures an Ilford 204 filter was interposed to remove stray light of short wavelength. Finally,

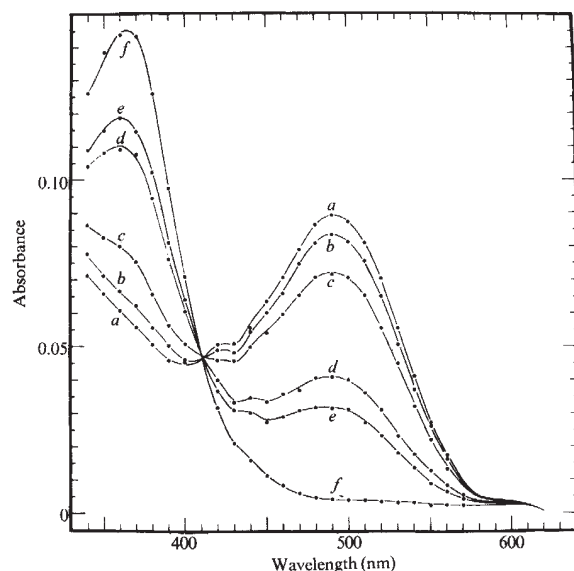


Fig. 1 Partial bleaching experiment on the extract of the panda visual pigment. Curve *a*, original absorbance spectrum, curves *b*, *c*, *d* and *e*, after exposures to 620-nm (*b* and *c*) and 600-nm lights (*d* and *e*), and curve *f*, after terminal exposure to white light, as described in text. As a test of stability the measurements were made at 20-nm intervals, first from 340–620 nm, then from 610–350 nm, then from 350–610 nm, and finally from 620–340 nm. ●, Mean measurements. The extract was stable at all stages.

the bleaching of the extract was completed by 15 min exposure to white light which had passed through a Wratten #15 filter.

When the overall change in absorbance (Fig. 1*a–f*) is plotted, a difference spectrum normal for a retinal-based pigment in the presence of hydroxylamine is obtained. The maximum loss of absorbance, $\Delta A_{\max}$, was 0.0850 (at 491.9 nm) and the maximum gain was 0.0875 (at about 367 nm).

Further extracts were similarly made from the already-extracted outer segments, and from the retinal debris remaining after flotation of the outer segments. These extracts, when bleached in the presence of hydroxylamine, yielded similar difference spectra but of much smaller $\Delta A_{\max}$, namely 0.0155 and 0.0070 respectively. Thus the total amount of extractable photosensitive pigment in the eye was equivalent to 0.525 ml of extract of $\Delta D_{\max} = 0.1075$ per cm.

The curves in Fig. 1 seem to represent the bleaching of a homogeneous pigment, for they all cross at nearly the same wavelength (410 nm) and the changes from stage to stage seem similar. This impression is not correct, however, as is found on plotting the individual difference spectra *a–b*, *b–c*, . . . , and comparing them. The positive portions of all the difference spectra could be closely matched to nomogram curves for retinal-based pigments. Seven estimates for $\lambda_{\max}$ were obtained by drawing smooth curves through each set of points, reading the wavelengths at which the absorbance change was 90, 80, . . . 30% of the maximum, and comparing with the nomogram. The means of these are listed in Table 1.

Table 1 shows that, apart from the first, very small, instalment of bleaching (*a–b*) which had $\lambda_{\max} = 496.9$ nm, the difference spectra for the long-wave bleachings were nearly constant, $\lambda_{\max}$ ranging from 493.7 nm for the *b–c* change to 492.4 nm for *d–e*. But the final bleach *e–f*, caused by exposure to white light, showed a distinct change of $\lambda_{\max}$ to 488.4 nm. To a first approximation, therefore, we can conclude that the panda extract contained two main components, one sensitive to red light, and a residue bleached by the white light. The difference spectra for these two components are given in Fig. 2. The spectra show that the photoproduct is identical in each case, with maximum absorbance at about 367 nm. This position is characteristic of the oxime of retinal, and indicates that both components are based on retinal (retinene₁).

Table 1 The Relative Amounts of Pigments 494 and 487 Bleached in Various Instalments

Change	Bleaching wavelength (nm)	$\lambda_{\max}^{\dagger}$ (nm)	$\Delta A_{\max}$	Pigment 494	Pigment 487
<i>a–b</i>	620	496.9	0.0060	*	(0.0000)
<i>b–c</i>	620	493.7	0.0113	0.0108	0.0005
<i>c–d</i>	600	493.4	0.0309	0.0283	0.0026
<i>d–e</i>	600	492.4	0.0093	0.0072	0.0021
<i>e–f</i>	white	488.4	0.0277	0.0055	0.0222
<i>a–e</i>	620–600	493.7	0.0574	*	*
<i>a–f</i>	—	491.9	0.0850	*	*
<i>b–f</i>	—	491.4	0.0791	0.0497	0.0294
<i>c–f</i>	—	491.1	0.0680	0.0398	0.0282
<i>d–f</i>	—	489.2	0.0370	0.0116	0.0254

* Bleachings that include the *a–b* instalment cannot be calculated because of the presence of the trace of very red-sensitive pigment (see text).

$\dagger$ These values are probably correct in an absolute sense only to ± 0.5 nm.

The difference spectra (Fig. 2) for the two components are not “pure”, because each is contaminated by the other. From Table 1, which shows the $\lambda_{\max}$ and the magnitude ($\Delta A_{\max}$) of the successive bleachings, it is seen that the red-sensitive component, excluding *a–b*, has $\lambda_{\max}$ in the region 494–493 nm. The effect of red light on the insensitive component is clearly slight, and I conclude that the major part of the red-sensitive component, *a–e*, consists of a pigment of $\lambda_{\max} = 494$ nm. This is 0.6 nm higher than for the *c–d* instalment and 1.6 nm higher than for *d–e*, in roughly inverse proportion to the size of these instalments and is consistent with the fact that “contamination” from the insensitive component is slight and virtually the same for both. In addition, as shown by the *a–b* instalment, there may be a trace of very sensitive pigment present, having $\lambda_{\max}$ longer (probably much longer) than 496.9 nm.

To obtain the $\lambda_{\max}$ of the insensitive component an estimate of how much of the final change (*e–f* in Fig. 1) was due to pigment 494 remaining from the earlier bleachings has to be made. A close estimate can be made by considering the effects of the two identical 600-nm exposures that caused the changes

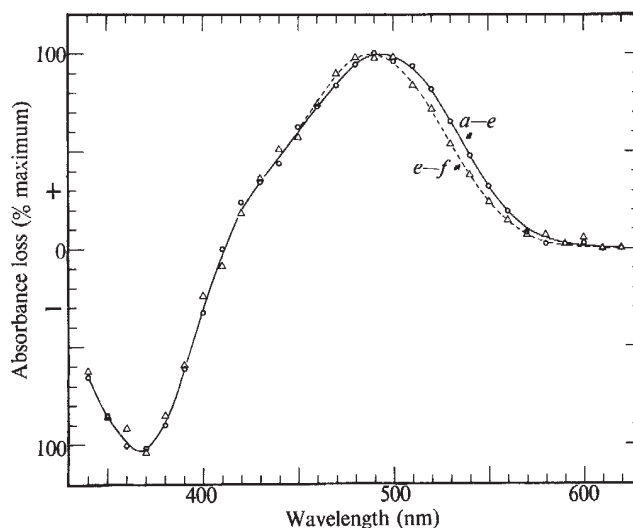


Fig. 2 Normalized difference spectra of the two photosensitive components in the panda extract. Curve through circles, constructed from the change *a–e* in Fig. 1, shows the component bleached by 620–600-nm lights; curve through triangles, constructed from the change *e–f* in Fig. 1, shows the residue from the earlier exposures that was bleached by white light. Note that the negative limb of each difference spectrum is identical and is maximal at about 367 nm, which is characteristic of retinal oxime and indicates that both components are based on retinal (retinene₁).

c-d and *d-e* in Fig. 1. In both cases these were mainly as a result of bleaching of the 494 pigment. In the first the absorbance loss was 0.0309 at maximum, and in the second 0.0093. Assuming that these values represent equal fractions of the pigment 494 present at the outset of each bleaching

$$0.0309/0.0402 + x = 0.0093/0.0093 + x$$

where x , the residue of pigment 494 left after the second bleach, is calculated to be 0.0040. The uncontaminated difference spectrum for the insensitive component can thus be obtained by subtracting the difference spectrum of pigment 494, scaled to a $\Delta A_{\max}$ of 0.0040 from the *e-f* curve of Fig. 2. The result of this operation is to shift the $\lambda_{\max}$ from 488.4 nm to 487.0 nm.

When two pigments (both fitting the A_1 nomogram) have $\lambda_{\max}$ as close together as 494 and 487 nm, there is an almost linear relation between the $\lambda_{\max}$ of any mixture of them and its composition: a 7:30 mixture would have $\lambda_{\max} = 491.9$ nm, and so on.

Table 1 shows the amounts ($\Delta A_{\max}$) of pigments 494 and 487 calculated in this way for some of the fifteen different spectra that can be constructed from the data of Fig. 1. From the table it is seen that the proportion of pigment 487₁ in the extract is about 0.028/0.085, that is, one third. The remaining two thirds consist almost wholly of pigment 494₁.

When, as in the present experiments, the optical density at the bleaching wavelength is small (≤ 0.05), the bleaching kinetics of a visual pigment can be expressed by the simple equation²

$$\log_e c_0/c_t = \alpha \gamma I_\lambda t$$

where c_0 and c_t are the pigment concentrations initially and after a time t , $\alpha \gamma$ is the photosensitivity, and I_λ is the light intensity per unit area. When a low-density mixture of two pigments is exposed to a bleaching light, each pigment bleaches independently, and then

$$\log p_1/\log p_2 = (\alpha_1 \gamma_1/\alpha_2 \gamma_2) \lambda$$

where the subscripts 1 and 2 refer to the two different pigments, and p is written for c_t/c_0 . In other words, the ratio of the photosensitivities is given by the ratio of the logarithms of the proportions remaining after a bleach.

From Table 1 it can be calculated that after the *c-d* bleach, 31% and 90% respectively of the pigments 494 and 487 remain. These values give a photosensitivity ratio at 600 nm of about 11. From the nomogram one would expect a 494 pigment to be only about 2.5 times as photosensitive as a 487 pigment at this wavelength. In an earlier experiment on the homogeneous 487.5-nm visual pigment of the red snapper, *Etelis marshi* (an Indian Ocean fish), the same bleaching conditions as for the present *c-d* exposure caused the bleaching of 34.5% of the pigment. The ratio of the photosensitivities of the panda 494 and red snapper 487.5 rhodopsins at 600 nm is about 2.5, as expected, confirming that the panda 487 pigment is insensitive.

All retinal-based visual pigments examined so far have been found to have very nearly the same photosensitivity at their respective absorbance maxima³. Hubbard and Kropf⁴, however, have shown in bleaching experiments with white light that the isorhodopsin (487 nm) derived from the opsin of cattle rhodopsin (499 nm) has only about one third of the photosensitivity of the parent pigment. The present result that the photosensitivity of the panda 487 pigment is only about one quarter of that of the panda 494 pigment suggests that it, too, is an isorhodopsin derived from the parent (494 nm) pigment. The addition of hydroxylamine to the panda extract and the fact that all the bleachings were carried out with nonisomerizing light, seem to preclude the possibility that the panda isorhodopsin was formed during the bleaching experiments. In any case, there was no evidence of any "regeneration", as all the spectra, which were measured twice over a period of about an hour, were quite stable. From this I suggest that the panda isorhodopsin may have been present from the outset in the extract. There seem to be two possibilities for its mode of

generation: it was either formed during the process of extraction or it was present in the retina.

As regards the first possibility, Rotmans, Daemen and Bonting⁵ have shown that a washed and concentrated suspension of bacteria (for example, *Proteus mirabilis*) caused a conversion of photolysed rhodopsin to isorhodopsin, and have suggested that a bacterial product can isomerize in darkness the all-*trans* retinal present in a photolysed rhodopsin preparation to the 9-*cis* isomer. Although the panda died (at 3.30 a.m.) under low illumination, it was not possible to remove the eye until about an hour and a half after death and, consequently, it is probable that the visual pigment was partly bleached. Moreover, as the retina was extracted overnight and then left to stand in darkness for another protracted period, it seems possible that some isorhodopsin could have been formed in the way described by Rotmans, Daemen and Bonting⁵.

As regards the second possibility, the presence of isorhodopsin in retinas has been reported^{6,7} after the eye has been exposed to very bright light. It seems unlikely that the panda isorhodopsin could have been formed in this way. The only other report of isorhodopsin in the retina is that of Kimura and Hosoya⁸ who found it in the retinas of hepatectomized toads and frogs.

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Bacterial Endotoxins in the Environment

DURING an evaluation of the *Limulus* lysate assay procedure¹⁻⁶ for the detection of Gram negative endotoxins, deionized water was found to be contaminated with endotoxins. Endotoxins were also present in samples of New Orleans tap water, which comes from deep levels of the Mississippi River. To appreciate the environmental distribution of endotoxins, studies were undertaken to compare the Gram negative endotoxin content of New Orleans water with that of other cities, as well as endotoxin distribution in various beverages and biological fluids.

The *Limulus* lysate technique of Levin and Bang¹ makes use of the principle that gelation of the amoebocyte lysate of the horseshoe crab (*Limulus polyphemus*) occurs in the presence of Gram negative endotoxins¹. The test is specific for Gram negative endotoxins⁴, and Reinhold and Fine⁴ and Yin *et al.*⁵ have reported that it can detect as little as 10^{-6} $\mu\text{g ml}^{-1}$ of endotoxin. The test is not positive when inactivated endotoxins are used⁵. Yin *et al.*⁵ showed that the *Limulus* lysate reacts with the lipopolysaccharide as well as lipid fraction of the endotoxin.

Tap water was obtained from various cities and placed

in sterile vials. Samples from distant cities were usually tested within a day of receipt. All other samples were analysed immediately. Control samples of sterile distilled water were similarly treated and all samples were analysed in duplicate. The lysate used was E-Toxate (Sigma Chemical Co., St Louis, Missouri) and all precautions associated with its use were taken¹⁻⁶. Studies were also carried out with lysate prepared in this laboratory.

Table 1 shows the endotoxin content of drinking water from eighteen cities, expressed qualitatively. When *Escherichia coli* endotoxin was used as laboratory standard, the *Limulus* test detected 0.001 μg of endotoxin in 0.1 ml of solution. This volume was routinely used in the assay procedure. Endotoxins were detected in sixteen of eighteen samples of drinking water.

Samples from cities such as Memphis, Tennessee and Kalamazoo, Michigan, which derive their water from Artesian wells were consistently negative. Galveston was an exception to this group. The reason for this discrepancy is not clear but might be associated with varying depths of the wells. Thus, in deep wells, it might be that endotoxins are removed from the water by the sieving action of soil. Samples from all other sources were positive.

Table 1 Endotoxin Content on Certain Municipal Drinking Waters, Mississippi River Water, the Gulf of Mexico and Adjacent Bays

Sample tested	Water source	Endo- toxin	Endotoxin concentration ($\mu\text{g ml}^{-1}$)
Drinking water			
Baltimore, Md.	Reservoirs	+	
Chicago, Ill.	Lake Michigan	+	
Denver, Colo.	Southplatt River and streams from Bear Creek	+	10
Galveston, Texas	Artesian wells	+	
Harrisburg, Pa.	Reservoir	+	
Hazleton, Pa.	Reservoir	+	
Kalamazoo, Mich.	Artesian well	-	
Knoxville, Tenn.	Fort Loudoun Lake	+	
Las Vegas, Nev.	Lake Mead (60%) Wells (40%)	+	
Little Rock, Arck.	Lake Maumelle	+	
Los Angeles, Cal.	Lake Winnoa Wells (12%) Colorado River (10%) Owen's River aqueduct from Mammoth Lake (77.7%)	+	
Memphis, Tenn.	Artesian well	-	
Mobile, Ala.	Reservoir	+	1
Nashville, Tenn.	Cumberland River	+	
New Orleans, La.	Mississippi River	+	1
Riverside, Ill.	Chicago water supply and 2 wells	+	
San Francisco, Cal.	Hetchy Reservoir	+	10
Washington, DC	Potomac River	+	
Surface water			
Barataria Bay, La.			20
Gulf of Mexico *			200
Little Dauphin Island Bay, Ala.			20
Mississippi River (surface levels)			130
Mississippi River (deep levels)			400
Mobile, Ala. (reservoir)			80

Samples were obtained between June and September 1972.

* Gulf of Mexico sample obtained off Grant Isle, La.

To determine the actual concentrations of endotoxins, samples were serially diluted and duplicate determinations carried out. Table 1 shows that tap water in New Orleans and Mobile contained 1 $\mu\text{g ml}^{-1}$ endotoxin compared with 10 $\mu\text{g ml}^{-1}$ in Denver and San Francisco samples. Surface water samples from the Mississippi River at New Orleans contained 128 μg endotoxin per ml whereas samples from deep levels (the source of tap water) contained approximately

400 $\mu\text{g ml}^{-1}$. The endotoxin content is obviously reduced during purification of the water for human use but it is not known at which stage in purification this occurs. Neither is it known whether the endotoxin content of source water parallels, on a seasonal basis, bacteriological analyses of untreated water, that is, highest levels during the summer.

A sample of water from the Gulf of Mexico contained 400 $\mu\text{g ml}^{-1}$ endotoxin whereas samples from bays off the coast of Louisiana and Alabama contained only 20 $\mu\text{g ml}^{-1}$. Carpenter *et al.*⁷ have reported the widespread presence of polystyrene beads in coastal waters of New England. These had rod-shaped Gram negative bacteria on their surface. This suggests that bacterial contamination of coastal waters could be one source of the endotoxin.

Tests carried out on biological fluids and beverages showed that chemically pure water, 5% dextrose, and sodium chloride solutions were negative for endotoxin. Random samples of beer, cola drinks and wine were also negative. One sample of locally obtained commercial bottled water was positive, but another brand, derived from a 2,000 foot deep Artesian well, was negative. Commercially bottled water from Mexico City was also negative. In contrast, Mexico City tap water had an endotoxin content of 800 $\mu\text{g ml}^{-1}$. Endotoxin was also found in milk samples from New Orleans. The range was 30 to 130 $\mu\text{g ml}^{-1}$ (Table 2). The endotoxin content rose sixteen-fold when milk was maintained at room temperature for 24 h with concomitant bacterial proliferation. No endotoxin alteration was observed when the samples were refrigerated.

In view of the presence of detectable amounts of endotoxins in laboratory deionized water, it seems that precautions should be taken over its use in various reagents or intravenous fluids. Where agents are to be administered parenterally, commercial samples of sterile saline, dextrose solutions and chemically pure water, which were shown to be devoid of endotoxin, should be chosen.

In spite of the toxic qualities of Gram negative bacterial lipopolysaccharides⁸, the presence of endotoxin in drinking water does not seem to constitute a health hazard to normal subjects in whom absorption is limited and adequate defence mechanisms are available for removal and inactivation of endotoxin. If minute quantities are absorbed, tolerance could be expected to develop⁹. If, however, absorption were increased as a result of increased permeability of the gastrointestinal tract, and if this were coupled with significant impairment in uptake and detoxification of endotoxins, a detrimental effect could be predicted.

Previous studies from this and other laboratories have shown that the intravenous administration of a single dose of lead acetate profoundly sensitizes animals to intravenously administered endotoxins⁹⁻¹⁴. It was therefore suggested that in certain conditions, sudden and unanticipated death might be related to interactions between endotoxins and environmental or dietary lead¹⁴. Blockade of the reticuloendothelial system or altered adrenal status can also increase sensitivity to endotoxin⁷.

Endotoxins also increase infectivity and mortality following bacterial infection, an event which seems to occur with high incidence in sudden infant death syndrome¹⁵. Rapid absorption of *E. coli* endotoxin has been demonstrated in piglets which then rapidly developed shock reactions^{16,17}. Whether reticuloendothelial dysfunction in the presence of endotoxin is a factor in acute unexplained death episodes has yet to be established.

Our observation of endotoxin in commercial milk samples and the increase in endotoxin content when milk is left at room temperature suggest that these may be contributory factors, as sudden infant death syndrome is rare in breast-fed infants¹⁵. In addition, enhanced gastrointestinal permeability in newborn infants^{18,19}, coupled with the possibility of induced impairment of the functional activity of the reticuloendothelial system, the site of endotoxin removal²⁰

and detoxification²¹, suggests that absorption of exogenously derived endotoxin and/or that produced by bacterial flora may, in certain conditions, possibly precipitate illness. Indeed, infants with or recovering from diarrhoea show enhanced absorption of intact protein^{18,19}. This is associated with increased alkalinity of the gastric contents, reflecting reduced hydrochloric acid production²². This would be expected to facilitate absorption of endotoxins in infants. Detection of endotoxin in drinking water and milk might possibly explain the recent observations of unexplained endotoxaemia in certain patients²³.

We are planning studies to evaluate the hypothesis that acute and unexplained death could be a result of altered reticuloendothelial function induced by lead or other agents, interacting with minute doses of either endogenously or exogenously derived endotoxin.

In view of the possible sources of endotoxin in surface water systems, and the simplicity as well as the sensitivity of the *Limulus* lysate test, it seems that this test could be effectively used not only to monitor water, milk and other beverages, but to establish standards of purity for biological solutions.

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Formation of Molecular Nitrogen by a Burning Cigarette

ALTHOUGH the total nitrogen content of tobacco leaf is routinely determined, a respectable nitrogen balance between the amount of tobacco consumed and the amount of smoke produced by a burning cigarette has not been achieved. In our studies using ¹⁵N tracer techniques¹ we have been disappointed in our nitrogen balance, both with respect to total nitrogen and to total label. The possibility of generating molecular nitrogen during the smoking process thus seemed worthy of investigation. No reports in the literature indicate molecular nitrogen formation on smoking; in fact, Jarrell and de la Burde² have reported that none is generated and delivered to the mainstream. We report here the results of our investigation which show molecular nitrogen to be a major product in sidestream smoke.

The cigarettes used were smoked in a helium: oxygen (80:20) atmosphere in a closed smoking chamber¹. Puffs were taken at intervals of 1 min using a 35 ml syringe. The sidestream was passed through a series of traps (dry ice, highly activated charcoal, ascarite, silica No. 28) in order to remove interferences such as oxides of nitrogen, and then gas chromatographed on a 21 foot × 0.125 inch column which was packed with 40 × 80 mesh 'Polypak' 2 (Hewlett-Packard). Introduction of an 800° C copper packed furnace at the injection port scavenged the oxygen present without affecting the nitrogen produced and thus facilitated the measurements. In an alternative procedure, the effluent gas from an entire cigarette was led to a thermal conductivity detector for quantitative measurement.

The cigarettes treated with ¹⁵N were handmade from Kentucky Reference 1R1 tobacco (1.14 g per cigarette) which had been sprayed with aqueous solutions of glycine-¹⁵N (96.2 atom %), calcium nitrate-¹⁵N (95 atom %), potassium nitrate-¹⁵N (99 atom %) or sodium nitrate-¹⁵N (99 atom %). These additives were applied at the 2.0, 1.8, 1.5 and 1.8% N level respectively. The nitrogen generated from these cigarettes was collected on molecular sieves at liquid nitrogen temperatures and the ¹⁵N incorporation determined by mass spectroscopy. Sidestream ammonia was also monitored and ¹⁵N enrichments were determined after conversion to nitrogen with sodium hypobromite.

Table 1 Nitrogen and Ammonia from Sidestream Smoke

Cigarette	N ₂ (mg/ cigarette)	¹⁵ N (atom %*)	NH ₃ (mg/ cigarette)	¹⁵ N (atom %*)
Ky ref. 1R1	2.7	0.59	5.3	—
Burley	2.9	—	8.5	—
Bright	2.6	—	5.6	—
Turkish	3.8	—	6.4	—
Ky ref. + glycine- ¹⁵ N	6.9	26	13.8	48
Ky ref. + Ca(¹⁵ NO ₃) ₂	5.3	32	9.0	33
Ky ref. + K ¹⁵ NO ₃	4.1	38	8.3	35
Ky ref. + Na ¹⁵ NO ₃	5.9	51	10.2	42

* Calculated from the intensities of the *m/e* 28, 29, and 30 signals.

Table 1 shows that a significant amount of the nitrogen in the tobacco leaf is converted to molecular nitrogen in the smoking process. The nitrogen is delivered almost entirely to the sidestream; that is, only a trace (<10 µg per cigarette) could be detected in the mainstream. These findings help considerably with respect to a nitrogen balance and several observations are worthy of note. First, both ammoniacal (glycine) and nitrate nitrogen are efficiently converted to both

ammonia and to molecular nitrogen, both of which are delivered almost exclusively to the sidestream (SS). Second, there seems to be a constant relationship between ammonia and nitrogen delivered in all cigarettes (except burley) studied; that is, $\text{NH}_3/\text{N}_2 = 1.9 \pm 0.2$. This is valid over a rather wide range of ammonia deliveries. Third, the ratios of $^{15}\text{N}^{15}\text{N}$ to $^{15}\text{N}^{14}\text{N}$ of the nitrogen delivered in sidestream and the nitrogen produced from sidestream ammonia are essentially the same for the glycine-treated cigarette, but quite different in the nitrate cigarettes (Table 2).

Table 2 Ratios of $^{15}\text{N}^{15}\text{N}$ to $^{15}\text{N}^{14}\text{N}$

Cigarette	SS N_2	SS $\text{NH}_3 \rightarrow \text{N}_2$
Ky ref. + glycine ^{15}N	0.49	0.43
Ky ref. + $\text{Ca}(^{15}\text{NO}_3)_2$	0.95	0.24
Ky ref. + K^{15}NO_3	2.0	0.26
Ky ref. + $\text{Na}^{15}\text{NO}_3$	1.9	0.35

These observations are compatible with the operation of an ammonia route to nitrogen³. In the nitrate cigarettes, however, particularly the sodium and potassium nitrates, the labelled ammonia generated must not mix with ammonia from the tobacco before conversion to nitrogen in order to satisfy the third observation above. This implies precisely defined time-temperature relationships around the coal. Alternatively, nitrate cigarettes may have superimposed on the existing ammonia route mechanistic routes which do not involve ammonia, that is, conversion of NO (ref. 4), NO_2 and/or N_2O (ref. 5) to nitrogen, or reaction of NO_x with ammonia.

Further work involving these mechanistic pathways is in progress.

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Grammar of a Movement Sequence in Inbred Mice

DETAILED descriptions of mammalian movement sequences are extremely rare. Such descriptions are necessary if we are to understand how behaviour is organized. We have found hierarchical principles of organization by describing in detail the sequences of face grooming components in mice. Grooming in rodents is an easily elicited movement complex that has proved useful in a variety of studies of behavioural integration^{1,2}.

Sixteen adult DBA/2J mice were placed individually in a 5 cm × 8 cm × 12 cm high plastic container and filmed at 32 frames s⁻¹. The film was analysed frame by frame with a stop action projector. A single face grooming sequence of 2 or more seconds from each mouse was included in the analysis.

We have found seven distinct components of face grooming: (1) shimmy (H), rapid vibration of body; (2) circle (C), flurry of forelimbs below face; (3) lick (L), wetting forepaws with tongue; (4) overhand (O), large synchronous but asymmetric strokes of forelimbs over top of head; (5) parallel (P), excursions of both limbs in similar half moon trajectories on sides of snout; (6) single stroke (S), 10 s⁻¹ horizontal movements on side of face in which each limb alternately makes the larger excursion; (7) pause (N), momentary interruption of active movement, with forelimbs at chest height. A more detailed description of these components, with illustrations, is available². Face grooming frequently leads into body grooming (B) but may also be followed by other behaviour patterns^{1,2}.

The structure of face grooming sequences was assayed by information measures^{3–5}. Initial analyses concerned transitional probabilities between adjacent grooming components. When expressed logarithmically (base 2) the eight grooming components (seven face plus body) represent three bits of uncertainty if one assumes equal probabilities and random sequencing. This measure (H_0) means that one could then guess correctly which component would occur next a mean of one out of eight (2^3) times. The observed component probabilities (H_1) reduced uncertainty to 2.36 bits; that is, one could predict the next component with a mean accuracy of 1/5–1/6. Knowledge of the preceding component (H_2) reduced uncertainty to 1.79 bits; that is, predictability of 1/3–1/4.

While these data indicate some sequential structure they fail to convey the degree of order apparent to the human observer. One could obviously extend analyses to include longer sequences of components^{3–6} but this is computationally cumbersome and might obscure other patterns of organization^{2,6,7}. We report two such patterns here.

First, we asked whether duration of a given component varies and whether this variation is related to the relative probabilities of subsequent components. Traditional measures of sequential coupling ignore this temporal parameter^{2–7}. Our data indicate its importance. To illustrate, short licking (less than 1/3 s) was immediately followed by overhand 42% and circle 19% of the time ($N=48$), intermediate licking (1/3–2/3 s) by overhand 22% and circle 39% ($N=18$), and long licking (more than 2/3 s) by overhand 6% and circle 63% ($N=16$).

More important, traditional analyses fail to take into account possible higher order structuring that may alter the coupling of individual components of behaviour during the overall grooming sequence^{2,6,7}. We therefore looked for the existence of groupings or “units” made of varying combinations of component activities during different phases of the overall grooming bout.

Our analyses reveal that five such units can be classified (Fig. 1): unit 1, varying combinations of all components except single-stroke (S); unit 2, long periods (at least 0.8 s) of licking (L) which may be momentarily interrupted by circling (C) and pauses (N); unit 3, a series of repeated single-strokes interrupted by an occasional short lick and usually preceded by one or more parallels (P); unit 4, repeated overhands (O) with rare short licking interjected; unit 5, varying combinations of all components with the exception of circle and shimmy (H).

These units, a refinement of previous studies with C57/6J mice², were initially determined by eye and later supported by computer analyses using the Hadamard transform⁸. The units demonstrate that the probability and association of individual components are not stationary but shift in a systematic way during the grooming sequence. That is, given knowledge of a

previous component, one can predict the subsequent component with far greater accuracy if also told the unit in which the components occur. The predictability of sequencing among the face grooming units plus body grooming is itself better than 1/2 ($H_2 = 0.69$). Much of the remaining variability is due to body grooming, which, as shown in Fig. 1, occurs approximately equally often after units 3, 4 and 5. In the three transitions from units 3–5 a single overhand was observed after single-strokes. If this were interpreted as an abbreviated unit 4 (defined in terms of multiple overhands) then the transitions between face grooming components would be predictable with a probability of 1 ($H_2 = 0$). We prefer the cautious interpretation at present. Not all grooming units need be expressed during a given bout (for example many bouts start with unit 2), but the sequencing among units that do occur approaches zero uncertainty.

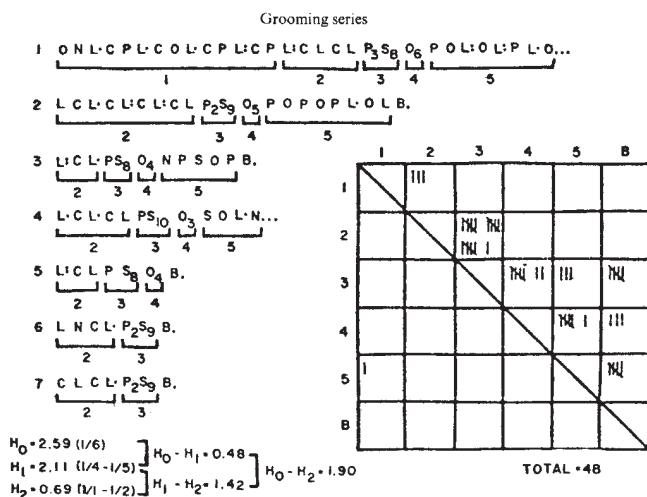


Fig. 1 Sample grooming sequences from seven mice. Grooming units 1–5 are illustrated by brackets beneath component letters. Repeated components are indicated by numerical subscripts. Licking duration indicated by L (<1/3 s), L- (1/3–2/3 s), L: (>2/3 s). In the grid of unit pairs, the preceding unit is indicated by the ordinate and the subsequent unit by the abscissa. Information measures are defined in the text.

Traditional sequential measures evaluate the mean structure and/or uncertainty throughout an entire sequence^{3,7}. By dissecting different subunits it is apparent that structure is highest (uncertainty lowest) during the middle portions of grooming (units 2–4), and that structure is lowest (uncertainty highest) at the beginning and end of the bout. Near the centre of a bout grooming also appears most “vigorous” to the human observer, and is more difficult to interrupt by extraneous stimulation.

These data provide a direct demonstration of the hierarchical structure in ongoing behaviour which has often been postulated by workers in both the behavioural and neurological sciences^{8,9}. We suggest that an analogy to human grammar, in which individual letters form different combinations in different words which in turn are sequentially arranged into phrases, may provide a more realistic model of control than attempts to explain coding simply on an element by element level. While similar proposals have been made before⁶, relevant quantitative data on an ongoing pattern of mammalian behaviour have not been available. The extent to which the different coding levels described here are subserved by separable biological mechanisms is currently being investigated in a series of genetic and neurological experiments in our laboratory.

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Asymmetry in Gorilla Skulls: Evidence of Lateralized Brain Function?

ASYMMETRY in mammalian skulls is a rare phenomenon. Differential tusk usage in elephants may result in slight skull asymmetry¹, and the Odontoceti (toothed whales) invariably show marked skull asymmetry caused by suppression of the nasal passage on one side. This is carried to an extreme in the Narwhal, in which a long tusk is almost always developed on the left side only.

Human skulls usually show a weak asymmetry, especially of the brain case. Hoadley and Pearson² measured the right and left internal lengths of the skull in 729 male Egyptian skulls belonging to the twenty-sixth to thirtieth Dynasty and found the mean length to be slightly greater on the right side than on the left (R : 171.0 mm, L : 170.1 mm). No other primate shows marked asymmetry except the gorilla. Coolidge³ noted and commented on this circumstance, observing that in “Coast gorillas” (*G. g. gorilla*) the right side of the skull was usually longer, while in “Mountain gorillas” (subspecies *beringei*, to which is now added subspecies *graueri*) the left side was usually longer.

We were struck by the asymmetry in Mountain gorillas (*G. g. beringei*, *sensu stricto*) while examining a collection of skulls at the camp of Miss Dian Fossey in the Virunga Volcanoes, Rwanda. Of eleven juvenile and adult skulls, four showed very marked asymmetry such that the left side was longer than the right. Only one of the four showed any sign of injury to the masticatory apparatus which might have caused such a condition. (A further skull, presented by Miss Fossey to the Duckworth Laboratory, showed the opposite type of asymmetry, but in this case it was quite clearly the result of extensive pathology of the masticatory musculature.)

We have looked again at Coolidge's data, supplementing them with our own observations on the skulls in the British Museum collection and the Fossey collection. Figure 1 shows the relative frequencies of differences between the length of the two sides of skulls (skull length measured from

the anteriormost point of the temporal fossa to the gnathion). For the Coast gorilla the mean difference, based on a sample of 138 skulls, is 0.1 mm to the right and is not significantly different from zero. For the other two subspecies taken together the mean difference, based on a sample of fifty-five skulls, is 2.5 mm to the left and is highly significant ($P < 0.001$ on a t test). Within the second group the bias to the left is greater in subspecies *beringei* than in subspecies *graueri* (mean differences of 2.7 mm and 1.8 mm, respectively), though in both subspecies the difference is significant ($P < 0.001$ and $P < 0.01$).

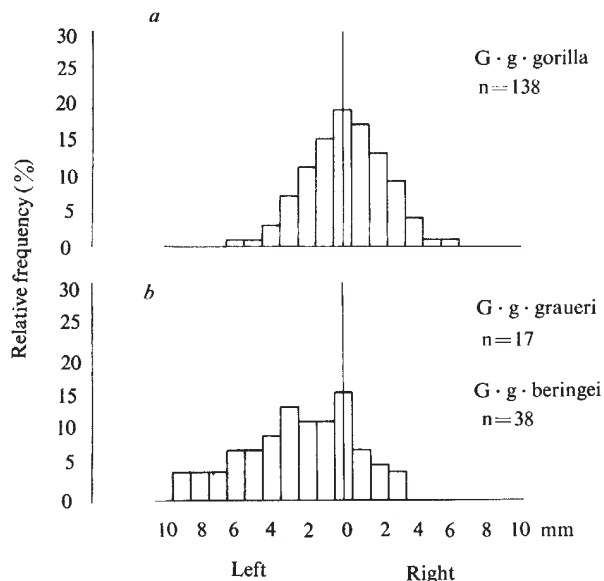


Fig. 1 Relative frequencies of differences between the lengths of the two sides of skulls of *G. g. gorilla*, *G. g. beringei* and *G. g. graueri*. Lengths were measured from the anteriormost point of the temporal fossa to the gnathion. a, *G. g. gorilla*, $n=138$; b, *G. g. graueri* ($n=17$) and *G. g. beringei* ($n=38$).

Besides looking at the general distribution of skull lengths we have found it useful to examine the incidence of gross skewing of the skull. Such "gross asymmetry" shows up unmistakably when there is a difference of 4 mm or more (roughly 2%) between the skull lengths of the two sides, and is accompanied by proportionate differences in other dimensions of the skull, an obvious lopsidedness of the sagittal crest, heavier toothwear on one side than the other, etc. Gross asymmetry is almost entirely restricted to true Mountain gorillas (*beringei*) (Table 1).

Table 1 Gross Asymmetry in *G. g. gorilla*, *G. g. graueri* and *G. g. beringei*

	Right side longer	Left side longer	No difference
<i>G. g. gorilla</i>	8	8	122
<i>G. g. graueri</i>	—	2	15
<i>G. g. beringei</i>	—	17	21

Gross asymmetry is seen to characterize the true Mountain gorilla to such a degree that almost as many specimens are affected as are not. Moreover, of the unaffected *graueri* sample, thirteen were "true" *graueri* from the Itombwe Mountains and the Utu lowlands, and two were from the equivocal Kayonza Forest population. Of the two affected

skulls, one was from Kayonza and the other from the other equivocal population, from Mt. Tshiaberimu.

Cerebral form has little direct effect on the shape of the skull, and we cannot argue with any confidence that these data on the Mountain gorilla reflect an asymmetry in the volume of the cerebral hemispheres. A plausible alternative would be that the skull asymmetry is a secondary consequence of an asymmetry in the use of the masticatory apparatus; for example, a tendency to chew more on the left side than on the right. Consistently one-sided behaviour, though almost unknown for other non-human primates, has been reported for the Mountain gorilla in another context by Schaller⁴. Schaller looked at "handedness" in the chest-beating display of eight Mountain gorillas in the field and found that the right hand was used first in fifty-nine out of seventy-two displays, all eight gorillas showing a right-hand preference. If left-sided chewing is the cause of skull asymmetry in Mountain gorillas, it might explain the absence of such asymmetry in Coast gorillas which have less strongly developed masticatory musculature. The existence of strong jaw muscles, *per se*, cannot be the explanation because orang-utans, which have equally well developed musculature to the Mountain gorilla, do not show comparable skull asymmetry.

Whether the asymmetry in the skull is directly related to anatomical asymmetry of the cerebral hemispheres, or whether it is merely a consequence of asymmetrical behaviour, it may be taken as suggestive evidence that there is asymmetry of function in the Mountain gorilla's brain. Among mammals, lateralization of brain function has so far been demonstrated only in man and is commonly assumed to be associated with the capacity for language.

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Perceptual Structure of the Necker Cube

STIMULUS variables influencing the perception of the third dimension and rate of perceived reversals¹⁻⁷ of the Necker cube (Fig. 1a-c) and the Mach book (Fig. 1d) have been examined, and although the Necker cube has been viewed as an ambiguous figure whose configuration and accompanying instructions usually limit the number of percepts to two⁴, few studies have asked what the observer perceives the figure to

be^{8,9}. We show here that the perceptual structure of the Necker cube is far more ambiguous than previously suggested.

We positioned a small directional marker such as an arrow on one of the faces of a reversible perspective drawing. From previous experience, the orientation of the directional marker appeared to change when the figure was seen to reverse but, in pilot studies, the reported changes in arrow orientation were not uniform. This variance between observers seemed to be due to differences in the way they perceived the structure of the Necker cube.

Twenty-one male and twenty female observers participated. The stimuli (Fig. 1) were constructed of tape (1 mm wide and fluorescent under ultraviolet light) attached to black paper. Directional markers were cut from the tape and attached to circular pieces of transparent plastic which could be laid over the figures.

Each observer was first shown a Necker cube figure and a standard set of descriptive terms was established. For example, in Fig. 1a, a right configuration occurred when the face marked *x* appeared closer and a left configuration when face *y* appeared closer. The observer, seated at a black surfaced table, viewed the test figures singly. Each observer selected his own viewing distance, which ranged from 30 to 40 cm. After obtaining a description of the perceived "orientation" and "location" of the directional marker when the test figure was seen in each of its configurations, the marker was removed and the observers were asked what they thought the drawings might represent. Half the observers viewed the test figures in ultraviolet light, thereby eliminating any possible influences from extrafigural stimuli, and the others under ordinary room illumination. By each illumination, half the observers used one directional marker and the rest used the other. The results were influenced neither by differences in illumination nor by the differences in directional markers.

The first drawing (Fig. 1a) can represent the effect obtained with about 50% of the observers. When the corner marked *x* was seen as closer than the corner marked *y* (a front right configuration), observers reported that the directional marker on the right side of Fig. 1a appeared to be on the inside right surface, and to point downwards. When *y* was seen closer (a left front configuration), the directional marker was seen to be on the outside right surface and pointing outwards towards the observer's left. If the directional marker was placed on the top of Fig. 1a and a front right configuration was seen, the marker was described as inside the upper surface and pointing away at a downward angle. When in the front left configuration, the marker was described as on the top of the upper surface and pointing toward the observer's left. This marked change in orientation of the directional marker will be called the Mason effect. Similar changes in the direction indicated by the marker are seen when the marker is placed on the other "sides" of the cube. The crucial condition seems to be that the marker indicates a direction approximately parallel to the obliques in the drawing, or in Fig. 1b and c a direction toward or away from the convergence point of the obliques. If the marker indicates a direction parallel to the vertical in the figures, no change is seen in the direction indicated by the marker when the figure reverses perspective.

The Mason effect in itself may be trivial because the orientation of the directional marker simply changes in the same way the perceived orientation of the drawing changes; but those observers who failed to report the Mason effect gave descriptions which, in general, resulted in a smaller change or no change in the perceived orientation of the directional markers. The most common response of this type (15% of the observers) can be described as a front surface response. For example, when Fig. 1a was seen in a front left configuration, these observers reported the marker on the right side as pointing towards them at a slight angle as though headed toward their left shoulder. When the figure was seen in the front right configuration, the directional marker was seen attached to the front surface of a cube-like object and pointed across the frontal

surface. Likewise, when seen in a front left configuration the marker on the top of Fig. 1a was seen on the upper surface of the figure pointing toward the observer's left, but when in the front right configuration the marker was seen on the "front" surface of the drawing pointing downward in the frontal plane. A few observers (about 10%) reported no change in orientation of the directional marker because they saw the marker attached to the back surface in the front left configuration and to the front surface in the other configuration. Thus, although the perceived spatial location changed, the orientation of the arrow did not.

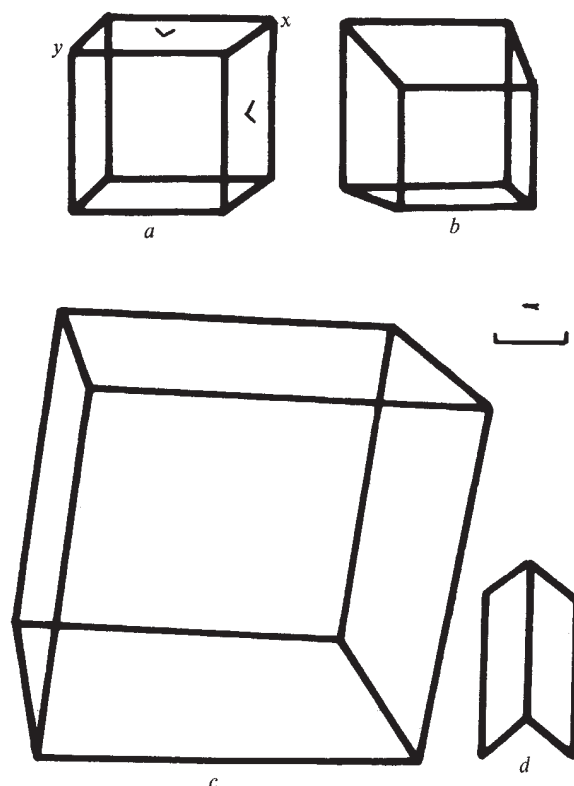


Fig. 1 Test stimuli used in study. The letters and directional markers on *a* are for purposes of description (see text). Observers were exposed to only one directional marker at a time. The other type of directional marker is seen just above the scale which indicates 1 cm.

The responses may be interpreted as arising from different perceptions of the Necker cube. We attempted to elucidate these perceptual structures by placing the directional markers at various orientations and locations on the figure. Those observers exhibiting a Mason effect probably perceived the structure as a container with an open front surface and with solid rear and side surfaces, as they always used terms like "inside" and "outside" and never reported the marker as lying on a front surface. Those observers giving front surface responses probably perceived a transparent six-sided cube of some sort, as individuals in this group sometimes spoke of the marker as on the inner surface of the faces, or occasionally described the marker as going through the cube. We describe this structure as a hollow cube even though we could not, in all cases, be certain that the cube was perceived as hollow. The perception of a solid cube was inferred in about 12% of the observers who failed, without prompting from the experimenters, to mention the possibility of the directional marker being inside the figure. In many cases these observers reported the arrow as not attached to a side of the figure, but rather at

right angles to the side, "trying to poke into the cube". The remainder of the observers (13%) gave responses compatible with a frame structure of some sort. This type of response involved no mention of sides. The directional markers were often described as non-attached, presumably floating, and were sometimes seen inside the framework.

Most observers consistently made responses which were compatible with one of the perceptual structures described above. Some observers (30%) did volunteer alternative descriptions of the location and orientation of the directional marker, perhaps because different figures suggested different perceptual structures. For example, Fig. 1a was often seen as a cube but Fig. 1b was more often reported as a tunnel or box of some kind. Observers were able to report alternatives once they became aware of these possibilities. When alternatives occurred observers always prefaced their responses with a clear statement that they were able to perceive a new structure. After the directional marker was removed and the observers asked to describe what the test figures represented to them, only about 50% of the observers used descriptions of the drawings which accorded with their description of the directional marker. The remaining observers gave verbal descriptions of the drawings which were inappropriate for the responses they had been making to the directional markers. For instance, several observers said the object was open at one end but consistently reported the directional marker to lay on this presumably non-existent surface. Therefore the observers' concept of the figure without the directional marker was often different from the structures inferred from their descriptions of the location and orientation of the marker. The important fact is that the Necker cube, with or without directional markers on its surface, is perceived differently by various observers. Moreover, when given explicit sets by the experimenter who described one or another of the structures, all but two observers gave descriptions of the directional marker compatible with the sets.

Responses to Fig. 1d produced few individual differences. The observers reported the expected changes in arrow orientation as the figure changed orientation. For example, when the directional marker pointed toward the spine of the Mach book observers reported that the marker pointed away from them when the spine was seen as farther away and toward them when the spine was seen as closer. Although this suggests that the perceptual structures were similar for all observers for figures like the Mach book, it seems clear that cube-like figures lead to alternative descriptions. Whether these differences in the perception of the figure relate to reversal rates and to measures of perceptual or cognitive styles¹⁰⁻¹² remains to be investigated.

That most observers are able to see the various possible alternative structures is not surprising, but the ease with which this is possible differs amongst the observers, which may suggest that the technique we used can also be applied to the study of personality variables such as dogmatism and rigidity¹³. The chief finding, that the structure of the Necker cube differs among observers, calls attention to a phenomenon that seems to have escaped notice since 1832 (ref. 14).

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Reference Abbreviations

FROM September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

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BOOK REVIEWS

Miserable Success

Where the Wasteland Ends: Politics and Transcendence in Post Industrial Society. By Theodore Rozsak. Pp. xxxiv + 492. (Faber and Faber: London, April 1973.) £3.75.

This is a book from the enemy camp: "... it is the culture of science from which we must liberate ourselves if we are to be free spirits" (page 73).

But it is a book so fair, so engaging, so disarming in its candid admission that the "counter culture" has little to offer but vague dreams and it is so well written that I can recommend it to my fellow-scientists, though when they have waded through his rich prose, embellished by much poetry, they will find little in it that was not said before, for example by Lewis Mumford or Aldous Huxley. Certainly, the following passage, on the last page but one, will appear familiar to many: "We 'progress' only towards technocratic elitism, affluent alienation, environmental blight, nuclear suicide. ... But there is another progress which is not a cheat and a folly; the progress that has always been possible at every moment of time. It goes by many names. St Bonaventura called it 'the journey to the mind of God'; the Buddha called it the eight-fold path; Lao Tzu called it 'finding the way'."

Perhaps only a great poet or a mystic could offer more, and Theodore Rozsak is not a poet, though he has a great affinity with poetry, as shown by his beautiful analyses of Blake (his favourite source), Wordsworth and Goethe. Neither is he a mystic; there is hardly an obscure or visionary passage in the whole book. Nor is he a revolutionary for revolution's sake like Marcuse. He is far too honest to suggest that all we have to do is to revolt, to smash up what he calls the "suave technocrat" system, and everything will come right.

Yet, unsatisfactory as Rozsak's message is when it comes to practical advice, it conveys in its many passionate chapters some deep truths about human nature. Man is not just a tool-making or symbol-making animal, he is also a myth-making animal. Tools and symbolic thought have led to the triumphs of technology, culminating in the "systems approach", which Rozsak fears and distrusts. They have also led

to the malaise in civilization, to the alienation which can be dulled but not cured by hard work and buzzing about in jet planes. But the beautiful myths, with which man used to round off his unsatisfactory real world, these have not kept pace with technical progress. Their decay has left a deep longing, which occasionally turns into hate and revolt. Rozsak, like most other honest intellectuals, recoils with terror at the prospect that science might satisfy man's irrational cravings by conditioning or by drugs.

If one takes this "misery of success" as seriously as it deserves to be taken, it comes almost as a relief that the advent of the perfect "suave" technocratic society, which satisfies all material cravings, is more remote than Rozsak seems to think. Though he is aware of the ecological limits, he appears to have been, at the time when he wrote this book, only a little time ago, like almost everybody else, ignorant of the grave fuel and energy crisis which is now about to hit the highly developed countries with the force of an express train. The crisis finds us so unprepared that it can hardly be overcome in less than two decades, and even then probably at the price of a temporary suspension of participatory democracy. Thus, for the next twenty years or so, we shall find ourselves in an archetypal situation for which our evolution has admirably prepared us; slipping down by our folly and then scrambling up again from the bottom. Perhaps, when this crisis is over, the "counter culture" will have something more positive to offer. Or perhaps the crisis will break our *hubris* sufficiently to make us ready for accepting alternatives to the affluent technocratic society.

DENNIS GABOR

Petrology

The Interpretation of Geological Phase Diagrams. By E. G. Ehlers. Pp. 280. (W. H. Freeman: San Francisco, 1973.) £5.40.

IGNEOUS and metamorphic petrology became a quantitative science when detailed mineralogical and chemical analytical studies of natural rocks were integrated with phase equilibrium ex-

perimentation. The technical demands of the latter have included pressures in excess of 5×10^6 N/m², temperatures in excess of 2,000 K and, frequently, control of a gas phase composition. It has taken nearly seven decades to establish a reasonably coherent picture of the sub-crustal and crustal genesis of the more important types of igneous melt and of the subsequent crystallization, and to define the physical chemistry of metamorphic recrystallization of rock masses.

Ehlers's book progresses from a discussion of high temperature equilibria at atmospheric pressure to topics such as systems at high pressures, systems containing water as a component, systems in which are encountered changes in oxidation state and systems with two volatile components. There is a great deal here, including a selective bibliography and a large number of illustrations, that offers welcome help to the student.

Many problems are posed by such a display of phase diagrams. For example, why does the system CaO-Al₂O₃-SiO₂ exhibit so many phase fields, and why are immiscible liquids encountered within it? Why does the SiO₂-Mg₂SiO₄-CaAl₂Si₂O₈ system exhibit non-ternary behaviour? The present volume does not attempt to answer such questions. It caters for the conventional geologist, who seldom asks for more than an empirical account of the chemical phenomena that he observes. Seemingly capricious behaviour, such as the incongruent melting of enstatite, can be made understandable, very simply, in terms of relationships between the free energies of liquid and of solid phases. It is a pity that an introductory account of such matters should be withheld.

The title of the book raises some misgivings. A whole range of geological aqueous equilibria has featured in recent studies¹, the relevant phase boundaries appearing as functions of variables such as *Eh* and *pH*. Low temperature solution chemistry lies outside the scope of the present volume, but of this the title conveys no hint.

E. D. LACY

¹ Garrels, R. M., and Christ, C. L., *Solutions, Minerals and Equilibria* (Harper and Row, New York, 1965).

Molecular Structure

The Electronic Structure of Molecules: Theory and Applications of Inorganic Chemistry. By G. Doggett. (*The International Encyclopedia of Physical Chemistry and Chemical Physics. Topic 4. Electronic Structure of Molecules. Volume 3.*) Pp. xvi + 172. (Pergamon: Oxford and New York, 1972.) £5.75.

DR GRAHAM DOGGETT is an experienced practitioner of theoretical chemistry, and he has written the present book to show how to calculate molecular wave functions, and how to use them in elucidating (or perhaps understanding) the geometrical shapes of molecules involving at least one atom heavier than neon. He writes clearly, and with authority. Moreover, he includes a discussion of several topics not often found in chemistry texts.

Indeed, this is not really an elementary text. It quite properly refers, time and again, to an earlier volume in the series, and assumes that the reader is familiar with simple ideas of *s,p* hybridization, electron pairing and straightforward theories of bonding. The real contribution of the book is in the role of *d* electrons. For example, in the chapter largely concerned with sulphur-containing molecules there is no reference to SH_2 , since this is similar to OH_2 . But there is a serious study of SF_6 and SF_4 , as well as PF_5 and PF_3 . (But why is there no account of sulphones and sulphoxides, where many interesting roles are played by the *d* electrons?)

Some readers will be glad to see a whole chapter devoted to the fluorides of xenon—a topic which has not yet found its way into most textbooks. But they may wonder why it was necessary to devote the greater part of the chapter to the valence-bond model, which—as the author quite properly says—is full of uncertainties—and only a smaller part to the molecular-orbital model.

The author shows a lively concern for the symmetry properties of wave functions, and the proper ways in which to incorporate chemical ideas of spin coupling. Indeed the first, and longest, chapter is almost wholly devoted to a useful discussion of how to set up molecular wave functions of appropriate spin and space symmetries. It is a pity that in the later chapters our mathematical techniques are so limited that full advantage cannot be taken of our knowledge of what a true wave function ought to look like. Perhaps in these matters the author is not quite fair to the use of Gaussian functions, whose very existence he does not specifically mention. But, all in all, this is a useful addition to the *Encyclopedia* of which it forms a part.

There is, however, one severe comment that must continue to be made,

though it has nothing to do with the author. This whole series is vastly too expensive. What student can afford £5.75 for a book of 172 pages, including the index? One could photocopy the whole affair for less than this. Why not paper covers, since surely we want to see as many copies sold as possible?

C. A. COULSON

Regeneration Miscellany

Regeneration in Lower Vertebrates and Invertebrates: II. By Margaret Egar. Pp. 167. (MSS Information Corporation: New York, January 1973.) \$15.

It is not quite clear what, or whom, this book is for. It reproduces eleven original articles published between 1967 and 1971 of which ten appeared in widely available journals. The provenance of the eleventh is not given. They are introduced by a very brief preface which, misleadingly, implies that regeneration in reptiles and in *Hydra* are among the topics covered. In fact all are on regeneration in amphibians—of limb, of tail or of lens. The cost is about 10 cents a page.

The articles themselves are specialized research reports, none is a review. They will therefore be of most interest to those whose interest has already been awakened. It is no criticism of the work reported to say that there seems to have been no particular object in selecting it for assembly in this way. The only editorial discretion that appears to have been exercised is the excision of the summary from some, but not all, of the articles.

To list the contents in full would be extravagant of space. The articles are by Egar and Singer, Tweedle, Lentz (limbs and their innervation), Tassara, Francoeur, Francoeur and Wilber (limbs) and Eisenberg-Zalik and Scott, Campbell and Jones, Reger, and Zalik and Scott (lens). They seem to me to range between the interesting and the very interesting.

D. R. NEWTH

Return of the Mind

Cognition in Learning and Memory. Edited by Lee W. Gregg. Pp. vii + 263. (Wiley: New York and London, 1972.)

ANY discipline which deals with complex phenomena has to simplify to make progress. In recent history experimental psychologists, especially in the USA, have frequently over-simplified both the experimental situations which were intended to give reproducible data and the theories which accounted for and were tested by the data. Even when man was the direct subject of study the empirical situations often remained remote from his everyday experience and the theories were little more than theories of the data, rather than being

theories of brain function. The new wave has the title cognitive psychology and it is well represented by this volume which is the proceedings of a conference held in 1969 at Carnegie-Mellon University. It is typified by willingness to reintroduce terms like "mental", "meaning" and "image", to accept introspection as data and, above all, to treat man as man has always treated the world by hypothesizing complex processes underlying the directly observable behaviour, utilizing whatever techniques and analogies were available. The computer has been a powerful force in this change as a means of analysing data, as a rich source of metaphor and as a means of simulating models which in a purely verbal description would be unintelligible and incomprehensible (see Gregg's introductory chapter).

The title of the book somewhat belies its contents, for there is as much discussion of visual imagery, comprehension and problem solving as there is of learning and memory. Such distinctions are, however, less valid than they used to be, because, for example, language comprehension is increasingly seen as using the same kinds of process as those employed in problem solving, and visual imagery clearly plays a part in experimental situations which used to be called verbal learning.

One illustration of the increase in complexity can be found in the treatment of memory. As little as ten years ago there were heated debates as to whether there were one or two kinds of memory. Wickelgren, in this volume, finds it necessary to distinguish between three or four kinds of memory trace (differentiated by the time constants of decay functions) for each of sensory (visual, auditory and textual), motor and conceptual modalities with four different kinds of association through which information is stored and retrieved. The notion of "association", by the way, is much more complex than that used in the past by stimulus-response psychologists, as it represents the operations of information processing systems and the complexities of the organization of our knowledge. Michon describes a couple of techniques used to elucidate such structures and Collins and Quillian summarize the work rising from a program by Quillian designed to comprehend written text by relating it to a semantic network filled with factual information. The model, although probably inappropriate in some regards, is complex in a plausible way. It does a reasonable job in predicting performance in tasks involving assigning a truth value to propositions such as "A canary is blue", and accounting for why it takes longer to answer "false" to "An almond has a fortune" than "A pecan has a castle". Other related theoretical developments can be found in *Organ-*

isation and Memory, edited by Tulving and Donaldson (Academic Press, 1972).

Three chapters, by Bower, Simon and Chase and Clark, discuss the use of visual imagery in a variety of tasks. The differences between these writers, lying as they do in the nature of the underlying abstract representation in such tasks, illustrate the sophistication of the current approach and provide a link to studies in psycholinguistics. In the remaining chapter, Calfee, Chapman and Venezky present an analysis of the skills a child requires if it is to learn to read.

In the entire volume there is little or no reference to or concern with the biological substrate of the postulated processes. This seems quite proper since psychological descriptions are necessarily different from physiological, the latter providing only limiting conditions for the former (such as time of operation), and justification for separation of particular functions (as with evidence from aphasia). There is still simplification, of course, but as Gregg's excellently produced volume shows, current psychological theories are interestingly powerful.

JOHN MORTON

Palaeomagnetism

Palaeomagnetism and Plate Tectonics. By M. W. McElhinny. Pp. x+358. (Cambridge University: London, February 1973.) £8.50; \$27.50.

ALTHOUGH shorter and cheaper texts on palaeomagnetism have appeared in recent years, this is the first attempt at a comprehensive presentation since Irving's *Palaeomagnetism and its Application to Geological and Geophysical Problems*. Hence it is natural to assess McElhinny's book against the standard set by Irving's. Since 1964 when Irving's book was published a new and fuller understanding of the evolution of the present day ocean basins has been worked out in terms of sea floor spreading and the concept of lithospheric "plates" is now widely accepted. So it is particularly appropriate that a new book on palaeomagnetism should also deal at some length with the geometry of "plate tectonics".

The book comprises about 280 pages of text followed by an appended list of palaeomagnetic data, a very comprehensive list of references and a good index. The first 150 pages of text contain an account of the method and principles of palaeomagnetism of which the essentials are clearly explained and the subject matter well balanced. The second half of the book is more in the nature of a review of the author's interpretation of palaeomagnetic data at this point in time.

Beginning with a chapter on the present geomagnetic field, the book

goes on briefly to deal with palaeosecular variations and palaeointensities. In the next chapter some elementary theory of magnetism is given and the nature of the commonest magnetic mineral series found in rocks and the physical principles of rock magnetism are described. Methods and techniques involved in carrying out a piece of palaeomagnetic work — methods of measurement, of collection of samples, cleaning procedures, field and laboratory stability tests and statistical method are outlined in another chapter.

In a chapter on reversals, well established phenomena such as the arguments for and against field reversals as opposed to self reversals of natural remanence and the polarity time scale going back to 24 m.y. ago deduced from palaeomagnetic studies of dated igneous rocks and of cores of sediment from the ocean floor are described. Then the reversal pattern over the longer time scale is discussed. Recently, at the Twenty-fourth International Geological Congress held in Montreal last year, a sub-commission of the International Commission on Stratigraphy was set up for the purpose of advising on a magnetostratigraphic nomenclature and advanced a recommended set of unit terms and hierarchies in palaeomagnetic stratigraphy. This unfortunately differs from that presented in McElhinny's figure 72 purporting to illustrate the reversal time scale in the Mesozoic in that, for example, his so-called "intervals" should be called "periods". More important, however, there is a strong feeling among palaeomagnetists against allotting special names (as McElhinny has done) to these magnetic chronostratigraphic units first until we are really certain of their existence and second because it is undesirable to burden the student with yet another set of stratigraphic names to learn.

In the later chapters, the Neogene and Quaternary palaeomagnetic evidence relevant to the establishment of the axial dipole field hypothesis is assembled and evidence for a second order displacement of the dipole from the geocentre by the order of a hundred kilometres is presented. The interrelationship between palaeoclimatic indicators and palaeomagnetic latitudes is dismissed in six pages in McElhinny's book whereas Irving devoted sixty pages to a discussion of this topic. This clearly reflects the different backgrounds of the two authors: McElhinny's background is physics while Irving's is geology, and this has had a notable influence on the form of the two books.

In the remainder of McElhinny's book, apparent polar wander curves for the various continents are described and then these data are synthesized and interpreted within the framework of the

concept of plate tectonics. The data are assembled, for the earlier part of the Phanerozoic, following a geographical pattern designed to demonstrate how Pangaea was formed by the fusion of pre-existing continental blocks.

Those who already have Irving's book and who wish to update their knowledge of the subject would find it cheaper to refer to one of the reviews of the subject presently available or which will undoubtedly appear from time to time in the near future. Those who wish to acquaint themselves with the situation in plate tectonics would be advised to consult a specialist book in this field such as Vacquier's recent publication. For those who do not own a book in palaeomagnetism, however, I recommend this as the best text available.

K. M. CREER

Experimental Epilepsy

Experimental Models of Epilepsy—a Manual for the Laboratory Worker. Edited by D. P. Purpura, J. Penry, D. B. Tower, D. M. Woodbury and R. D. Walter. Pp. 615. (Raven: New York. Distributed in the Eastern Hemisphere by North-Holland: Amsterdam, 1972.) Sfl.75; \$23.50.

IN compiling this volume dedicated to the memory of the late Don Esplin, the editors have had two stated goals. They have sought, on the one hand, to bring together in a single book a comprehensive description of the methods employed to produce various experimental models of epilepsy. To this end they have been more than successful, and the editors are to be congratulated. Twenty-four contributing experts in the field have described in detail the methods and techniques that they employ in models ranging from *in vitro*, isolated brain tissue to the light sensitive baboon.

Their second stated aim is that "the book be as critical in its approach as it is detailed in its methodology". Here the editors have fallen short of the mark. Specifically, the book suffers from two serious deficits, one a consequence of the other. They seem particularly grievous because the editors direct this book primarily to the "young investigator". The chief drawback of this volume is that it lacks a chapter devoted to a critical overview of the comparative relevance of particular models as they relate to particular experimental ends.

With the exception, perhaps, of the use of induced epilepsy as an "interfering technique" in the study of learning and memory paradigms, most experimental studies of epilepsy have at least as their covert intention a better understanding of human epilepsy. Perhaps the chapter which comes closest to filling this void is that of Jasper. In his chapter he briefly discusses the applicability of

various models to various types of human epilepsy. However, I must concur in Jasper's own view that a detailed discussion of the technical and methodological aspects of various experimental epileptic models as applied to various problems in epilepsy is clearly beyond the scope of this chapter. It remains necessary, therefore, for the reader, who does not already possess broad knowledge in his field, to read the entire volume and fend for himself. Thus as a manual this volume will probably be difficult for the neophyte epileptologist to use. The other major defect which results in part at least from this lack of a critical overview is the limited index. For example, a unique model of epilepsy, like the "kindling effect" of Goddard, is not to be found in the index, although it is referred to in both chapter 4 and chapter 6. This limited index will make it difficult for the inexperienced investigator to readily find the wealth of material contained in this book.

These shortcomings notwithstanding, this book should be a valuable addition to the library of all who are interested in experimental epilepsy. IRA SHERWIN

Uses of Graph Theory

Graph Theory and Computing. Edited by Ronald C. Read. Pp. xiv+329. (Academic: New York and London, October 1972.) \$17.50.

THIS book consists of eighteen papers presented at an International Conference held at the University of Waterloo, Canada, most of them research papers presented by expert workers in the combined fields of computing and graph theory. An excellent feature of this book is that most of the papers are self-contained. The standard is very high and there are few errors, typographical or otherwise. The papers cover a wide spectrum of areas where graph theory and computing interface. In some papers, certain topics in the theory of automata, numerical analysis and operations research are investigated from a graph theoretic point of view, whilst in others computing techniques are applied to graph-theoretic problems.

Areas not covered in the book are, among others, web grammars and their characterizations by planar graphs, analysis of loops in a program and segmentation of programs using graph-theoretic concepts and techniques, and finally graph theory applied to the analysis of complex information structures. This is to be expected, however, for graph theory and its application in computing is a rapidly expanding field.

The book will appeal to pure graph theorists, computer scientists, modern applied mathematicians and possibly electrical engineers. Any research worker in these fields is well advised to

keep a copy of this book. Additionally, listed in the book are various unsolved problems, which can easily form the core of further research. All in all, the book is an admirable endeavour in bringing together the various disparate fields where graph theory, matrix theory and computing are interwoven.

G. LOIZOU

Equilibrium Analysis

Mathematical Methods in Theoretical Economics. By Erwin Klein. Pp. xix+388. (Academic: London and New York, February 1973.) \$16.50.

THIS is an early volume in a new series on mathematical economics, one of several very welcome series now running. The title over-sells the contents and a better description is provided by the subtitle: "Topological and Vector Space Foundations of Equilibrium Analysis".

There are three broad and somewhat overlapping areas of mathematical methodology currently cultivated by economists: analysis including real and complex variable, difference, differential and mixed equations; linear algebra and theory of linear models; game theory, programming and control theory. Economic theorists take fairly naturally to the set-theoretic and mathematico-logical underpinning of mathematical methods and, indeed, they have developed some direct applications to economic models. Erwin Klein has here confined himself to this last topic and in so doing he has produced a clear and useful text for an advanced course in mathematics for economic theorists.

This said, it needs to be added, as the author himself notes in his preface, that the book comprises two short and separate texts on only distantly related subjects. In each case, a sequence of purely mathematical chapters is supplemented by a few economic examples and followed by a short but direct and systematic application to a basic economic problem.

The first of the two almost self-contained texts is on point-set topology as a basis of function theory (analysis). The topics discussed are wider in scope than this might suggest and include point-to-set as well as point-to-point mappings and fixed-point theory. The concluding application is a simplified version of Debreu's axiomatic approach to the problem of general economic equilibrium. The second text is a development of modern linear algebra on the basis of vector spaces and, of the many uses in economics, the one selected for brief exposition is Gale's version of the classic model of growth first constructed by von Neumann.

The first of these texts may be without a serious competitor as a course

for economists but the second faces quite stiff competition, for example, from Yaari's recent *Linear Algebra for Social Sciences*, a good deal simpler than Klein though less wide in scope. Klein's writing is clear and precise; a list of terms and notations would, however, make for even easier reading. Of the few detected slips the most serious is at the top of page 309 where the conditions for maxima and minima are in fact sufficient only. Necessary conditions are easily written by substituting $\leq$ for $<$ (and $\geq$ for $>$); necessary and sufficient conditions involve more than second-order terms.

R. G. D. ALLEN

Rate Processes

Advances in Linear Free Energy Relationships. Edited by N. B. Chapman and J. Shoeter. Pp. xiv+486. (Plenum: London and New York, September 1972.) \$28.

"To my mind a particularly happy aspect of the existence of linear free energy relationships has been the proof it supplies that one need not suppose that the behaviour of nature is hopelessly complicated merely because one cannot find a theoretical reason for believing it to be otherwise". Thus Louis P. Hammett in a foreword to the present volume indicates the frame of mind in which he proposed the famous equation which bears his name. The value of linear free energy relationships "... should not be obscured either by the fact that there are always small seeming deviations from the relationships ... or by the over-optimism with which relationships have sometimes been reported or interpreted".

The late Sir Christopher Ingold said that a theory could be simple and inaccurate, or complex and accurate, but should never be complex and inaccurate. The strength of the original Hammett equation (1) lay in the fact of its simplicity. For a reaction

$$\log k/k_0 = \rho\sigma \quad (1)$$

(characterized by ρ), the relative rate of a substituted compound, as compared with the unsubstituted species, depended only upon σ , a property of the substituent. The present volume examines the subsequent developments of equation (1) in many diverse fields ranging from drug action to mass spectrometry. As is not uncommon these days, the volume is composed of chapters written by experts in the various fields, reviewing their particular areas. The extent to which this has been successful is to some degree evidenced by the 2,500-odd references to be found in the volume.

This book will be of interest to all concerned with the physical-organic approach to rate processes.

ALLAN MACCOLL

CORRESPONDENCE

Landing on Mars

SIR,—After more than a year of arduous study, the Viking landing site selection team has chosen four tentative landing sites on Mars for the summer of 1976, when it is hoped two NASA Viking landers will be set down on the planet. These locales were chosen with considerable attention to site safety and likely scientific return. As a participant in the selection team I can attest that a wide range of alternative views were considered in a very open manner. Our ability to predict surface properties on an unexplored planet, however, using photographs with at best 100 metre resolution, is clearly limited. The prime and backup sites for the A and B landers are as follows: A1 (19.5° N, 34° W): Chryse; A2 (20° N, 252° W): Tritonis Lacus; B1 (44.3° N, 10° W): Cydonia; B2 (44.2° N, 110° W): Alba.

It is of interest to imagine the circumstances reversed, and four landing sites with precisely these planetocentric coordinates chosen on the planet Earth for investigation by hypothetical spacecraft from somewhere else. The comparable landing sites are these: A1 (19.5° N, 35° W): Atlantic Ocean, Cape Verde Basin; A2 (20° N, 108° E): Gulf of Tonkin, 0.2° S of Nightingale I.; B1 (44.3° N, 10° W): North Atlantic, 9° W of Mizan (near Bordeaux); B2 (44.2° N, 110° W): Wyoming, SE corner of Yellowstone National Park.

Corresponding nicely to the ratio of water to land area on the planet Earth, we see that three of the four landing sites are in the ocean. While occasional events may be detected during a short stay in some of these places—site A1 is in the Canary Current used by 16th century sailing vessels travelling to the “New World”, and naval activity is not entirely unknown in the Gulf of Tonkin—the probability of encountering such events during a brief flotation would be low. But site B2 is near a tributary of the Yellowstone River, adjacent to some of the most spectacular geology and most interesting biology on our planet.

It is sobering to imagine the picture of the planet Earth that would be communicated to a space-faring society on another planet after a brief inspection of these locales. Were two spacecraft to be landed in sites A1, A2, or B1, they would probably sink out of sight into the ocean and probably never be noticed by the inhabitants of the planet Earth. The B2 site would be rather unrepresentative of the geology of the

Earth, but would provide a substantial chance of detecting indigenous life and a better than average chance of detecting intelligent life. A reasonable strategy of extraterrestrial exploration of the Earth—noting the separation of surface features into “bright” and “dark” areas—might be to land one spacecraft at Yellowstone and the other in the Atlantic Ocean. Yellowstone might be selected because of its apparent high interest at 100 metre resolution for geologists and the Atlantic Ocean might be chosen as “safe” because of its smooth appearance at 100 metre resolution.

If we imagine a Mars as heterogeneous as the Earth—and the Mariner 9 investigations surely point in this direction—the conclusion is clearly that more than a few landers are necessary for an adequate *in situ* characterization of the principal features of the Martian environment.

Yours faithfully,

CARL SAGAN

Laboratory for Planetary Studies,
Cornell University

Entry to EMBO

SIR,—The setting up of the European Molecular Biology Laboratory in Heidelberg (*Nature*, **243**, 125; 1973) provides a splendid opportunity to bring together a group of stimulating and creative minds.

However, it is well known that scientists are at their most stimulating and creative before reaching the age of about 35, and if the staff of the EMBO laboratory are to be international, then this will impose a severe restriction on the number of non-British members. The reason for this is quite simple. Presumably the staff will be drawn from universities, and will require a PhD to conduct independent research. In this case, our average English molecular biologist would be around 25, but our fellow mainland European would be nearer 35.

This is not meant as an argument against cooperation. On the contrary, it is a plea to seize an opportunity to break down anachronistic barriers, and to spend the millions of dollars allocated to EMBO with more regard to a fertile rather than a sterile generation.

Yours faithfully,

H. J. LEESE

Lerchenrain 19,
8046, Zürich

Errata

THE contents entry for the article “Mutual Antagonism between Botulinum Toxin and α -Bungarotoxin” (*Nature*, **243**, 166; 1973) is incorrect and should read “Toxins—Bacterial botulinum toxin and snake venom toxin α -bungarotoxin act at different sites—...”.

IN the article “Increased Yield through Correction of Sulphur Deficiency in Ryegrass Exposed to Sulphur Dioxide” by D. W. Cowling, L. H. P. Jones and D. R. Lockyer (*Nature*, **243**, 479; 1973) the following correction should be made: paragraph 3, line 11, “... potassium simply as KCl plus KH_2PO_4 ”.

Announcements

International Meetings

August 6–8, **Thermophysical Properties**. (Symposium Chairman, School of Mechanical Engineering and Thermophysical Properties Research Center, Purdue University, Lafayette, Indiana, USA 47906.)

August 13–16, **Sixth Annual Meeting of the Society for Study of Reproduction**. (Claude Cruse, 113 N. Neil Street, Champaign, Illinois 61820.)

August 13–17, **45th ANZAAS Congress**. (Mrs Dulcie Stretton, ANZAAS Congress Executive Officer, University of Western Australia, Nedlands, W.A. 6009.)

August 14–16, **Microwave Semiconductor Devices, Circuits and Applications**. (Professor Lester F. Eastman, Cornell School of Electrical Engineering, Phillips Hall, Ithaca, NY 14850.)

August 15–17, **17th Canadian High Polymer Forum**. (17th Canadian High Polymer Forum, Pharmaceutical Chemistry Division, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2.)

August 19–23, **The Role of the Cell Surface Interactions in Development and Differentiation**. (VIIth International Congress, Department of Anatomy, University of Montreal, P.O. Box 6128, Montreal 101, Canada.)

August 19–24, **Developmental Biology**. (VIIth International Congress of Developmental Biology, c/o Department of Anatomy, University of Montreal, P.O. Box 6128, Montreal 101, Canada.)

August 20–24, **Fifth Meeting of the North West European Microbiological Group.** (5th Meeting of the North West European Microbiological Group, Bergen Congress Service Ltd, P.O. Box 909, N-5001 Bergen, Norway.)

August 20–24, **British Association 125th Annual Meeting.** (British Association for the Advancement of Science, Fortress House, 23 Savile Row, London W1X 2AA.)

August 20–25, **Third International Conference of the Strength of Metals and Alloys.** (Third International Conference of the Strength of Metals and Alloys, Institute of Metals, 17 Belgrave Square, London SW1 8PU.)

August 20–25, **Third International Gondwana Symposium.** (The Australian Academy of Science, P.O. Box 216, Civic Square, Canberra, ACT 2608, Australia.)

August 20–29, **XIIIth International Congress of Genetics.** (Office of the Secretary General XIII I.C.G., Department of Genetics, 345 Mulford Hall, University of California, Berkeley, California, USA 94720.)

August 21–31, **The Physics and Mathematics of the Nervous System.** (The Director, Institute for Information Sciences, University of Tübingen, 74 Tübingen, Köstlinstr. 6, Fed. Rep. Germany.)

August 22, **Prospecting in Areas of Glacial Terrain, Joint Meeting and Field Visits, Norway.** (The Secretary, Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR.)

August 22–24, **APL Congress 73.** (Secretariat, Helle Hornung, Brøndbyøster Boulevard 22, 2650 Hvidovre, Denmark.)

August 22–24, **Denver Conference on Application of X-ray Analysis.** (Dr C. O.

Ruud, Metallurgy and Materials Science Division, University of Denver, Denver, Colorado 80210.)

August 27–29, **International Conference on Comparative Virology.** (Professor E. Kurstak, Department of Microbiology and Immunology, Faculty of Medicine, University of Montreal, P.O. Box 66128, Montreal 101, Quebec, Canada.)

August 27–30, **Third International Conference on High Voltage Electron Microscopy.** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford OX1 3HA.)

August 27–31, **Nuclear Techniques in Comparative Studies of Food and Environmental Contamination.** (Secretary FAO/AEA/WHO, c/o International Energy Agency, PO Box 590, Kaerntnerring III, A-1011 Vienna, Austria.)

August 27–31, **Van der Waals Centennial Conference on Statistical Mechanics.** (Van der Waals-Laboratorium, Valckenierstraat 67, Amsterdam-C., The Netherlands.)

August 29–31, **Design Activity International Conference.** (Dr T. W. Maver, Programme Director, Abacus, University of Strathclyde, Glasgow G4 0NG.)

Reports and Publications

not included in the Monthly Books Supplement
Great Britain and Ireland

The Physics Exhibition Handbook 1973: 57th Exhibition of Scientific Instruments and Apparatus, Earls Court, London, 9–13 April 1973. Pp. xviii+176+12. (London and Bristol: The Institute of Physics, 1973.) [193]

University of Oxford. Ninth Annual Report of the Delegates of the Science Area for the year ending 31 July 1972. (Supplement No. 3 to the *University Gazette*, Vol. ciii, March 1973.) Pp. 141. (Oxford: The University, 1973.) 75p. [193]

The Fair Housing Experiment: Community Relations Councils and the Housing of Minority Groups. By Jane Perry. Pp. 53. (London: Political and Economic Planning, 1973.) £1.80. [193]

Symposium on Diet as a Risk Factor in Cardiovascular Disease, held at the University of Surrey, Guildford, 6/7 July 1972. (Nutrition Society Symposia Reprint Series, No. 2.) Pp. 297–362. £1; \$2.05. Symposium on the Influence of Amino Acid Supply on Polynucleotide and Protein Metabolism, held at the School of Agriculture, University of Aberdeen, 1 June 1972. (Nutrition Society Symposia Reprint Series No. 3.) Pp. 149–295. 75p; \$2. (London: The Nutrition Society, 2 Queen Anne Street, W1, 1972.) [193]

The Zoological Record, 1969, Vol. 106, Section 6b: Vermes. Compiled by J. W. Coles. Pp. 76. (London: The Zoological Society of London, 1973.) £3.50; \$8.50. [203]

European Economic Community Preferences: Free Trade Agreements between the EEC and the EFTA Countries. (Notice No. 810.) Pp. 136. (London: Her Majesty's Customs and Excise, 1973.) [203]

The Ciba Foundation for the Promotion of International Cooperation in Medical and Chemical Research. 1972 Report. Pp. 64. (London: The Ciba Foundation, 1973.) [213]

Agricultural Research Council. Meat Research Institute Memorandum No. 1: Some Technical Repercussions on the Meat Industry of EEC Entry. By C. Bailey, C. L. Cutting and A. G. Kitchell. Pp. 5. (Langford, Bristol: Meat Research Institute, 1972.) [213]

Imperial Cancer Research Fund. Annual Report and Accounts 1972. Pp. 76. (London: Imperial Cancer Research Fund, 1973.) [223]

The University of Leeds. Annual Report 1971/1972. Pp. 171. (Leeds: The University, 1973.) [233]

Report of the Population Panel. (Cmd. 5258.) Pp. xi+135. (London: HMSO, 1973.) 90p net. [233]

Department of the Environment. Welsh Office. Local Government Financial Statistics, England and Wales, 1970/1971. Pp. 58. (London: HMSO, 1973.) 70p net. [233]

Other Countries

Zenith Radio Corporation. 1972 Annual Report. Pp. 28. (Chicago: Zenith Radio Corporation, 1973.) [193]

Bulletin of the American Museum of Natural History. Vol. 149, Article 3: The Avifauna of the Kakamega Forest, Western Kenya, including a Bird Population Study. By Da' A. Zimmerman. Pp. 255–340. (New York: American Museum of Natural History, 1972.) \$3.40. [203]

United States Department of the Interior: Geological Survey. Professional Paper 755: Calorimeters for Heat of Solution and Low-Temperature Heat Capacity Measurements. By Richard A. Robie and Bruce S. Hemingway. Pp. iv+32. (Washington, DC: Government Printing Office, 1972.) 40 cents. [203]

Publications de l'Institut Orientaliste de Louvain. Expedition Philby-Ryckmans-Lippens en Arabie. Rock-Art in Central Arabia. 3. By Emmanuel Anati. Pp. 167. (Louvain-la-Neuve: Université Catholique de Louvain, Institut Orientaliste, 1972.) [223]

US Department of the Interior: Geological Survey. Bulletin 1372-B: Stratigraphic Nomenclature of Cambrian and Lower Ordovician Rocks of Easternmost Southern Arizona and Adjacent Westernmost New Mexico. By Philip T. Hayes. Pp. iii+21. 25 cents. Professional Paper 738: Silurian Rugose Corals of the Klamath Mountains Region, California. By Charles W. Merriam. Pp. iv+50+8 plates. \$1. (Washington, DC: Government Printing Office, 1972.) [223]

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